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World Distribution of Brook Trout, *Salvelinus fontinalis*

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During the past century, the endemic range of the brook trout, *Salvelinus fontinalis* (Mitchill), has been extended to include western North America and the continents of Europe, Asia, Africa, and South America. Water temperature appears to be the most important single factor limiting the geographic range, but adequate precipitation and suitable spawning areas are necessary also for the establishment of self-sustaining populations. It is improbable that, with the possible exception of Asia, the present range of the brook trout will be greatly extended through further attempts at introduction.

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INTRODUCTION

THE ENDEMIC RANGE of the brook or speckled trout, *Salvelinus fontinalis* (Mitchill), has, since 1872, been greatly extended to include waters in western North America, South America, Eurasia, Africa, and New Zealand.

The species, which early immigrants to eastern North America erroneously named a trout rather than a char because of a similarity to the European *Salmo trutta* Linnaeus (Rhead, 1902), was first described by Mitchill in 1814 from specimens captured near New York City (Jordan and Evermann, 1896). Although DeKay (1842) later assigned the generic *Baione* to the brook trout, the use of *Salvelinus* has persisted as it has for the Arctic char (*S. alpinus*) and the dolly varden (*S. malma*), these species being indigenous to the extreme northern and western parts, respectively, of North America. However, it is probable that the native ranges of *S. alpinus* and *S. fontinalis* overlap in the coastal streams between the Churchill and Nelson rivers on the west coast of Hudson Bay (Sprules, 1952).

Primarily because of differences in the external appearance of brook trout living in different waters, various specific names have been given to local representatives including *fontinalis*, *alleghaniensis*, *nigrescens*, *canadensis*, *hoodii*, *immaculatus*, *hudsonicus*, and *agassizi*. Rather spectacular differences in the appearance of the Aurora trout (*S. timagamiensis* (Henn and Rinkenbach)) has led to a more persistent recognition of this local form as a distinct species, but Vladykov (1954) suggests that it may be considered to be a colour variety of *S. fontinalis*. The American Fisheries Society (1960) now recognizes only *fontinalis* as the specific name for the brook trout.

The objectives of this paper are: (1) to consolidate and update knowledge on the native distribution of *S. fontinalis* (Mitchill); (2) to record attempts

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to introduce the species beyond its endemic range; and (3) to document the present world distribution of native and naturalized populations. From a consideration of the biology of the species and the ecological conditions where native and naturalized populations now exist, evidence is presented to identify those environmental conditions which affect the welfare of local populations and would seem to limit the potential world distribution of self-sustaining populations of the brook trout.

NATIVE RANGE

The westerly limit of the native range of the brook trout occurs in Manitoba (Fig. 1). Although believed until recently to extend northward to include only Kettle Rapids and the Weir and the Limestone rivers on the Nelson River system (Doan, 1958), Keleher and Kooyman (1957) found this species farther northward in the lower waters of the Seal, North Knife, South Knife, and Churchill rivers. Other native populations are present, also, near the eastern boundary of Manitoba in the Gods River system as far south as the Island Lake, Red Sucker, Stull, and Echoing rivers (Keleher and Kooyman, 1957; Benedictson, personal communication).

In northwestern Ontario, Ryder et al. (1964) collected brook trout in the Patricia District and upstream in the Severn and Fawn rivers as far as Big Trout Lake; also in the Winisk River as far as Winisk Lake; in the lower reaches of the Attawapiskat River; and in the Albany River at least to the Fort Hope–Miminiska region. Brook trout occur naturally in Lake Nipigon (Dymond, 1926) but are absent from both the Nelson River watershed of Ontario (Ryder et al., 1964) and Lake of the Woods (Evermann and Latimer, 1910). Although endemic populations are absent from southwestern Ontario (Urquhart, 1957), they exist in streams tributary to the Great Lakes (Scott, 1954) and northward to include the rivers flowing into James Bay (Vladykov, 1933) and the east coast of Hudson Bay at least to Povungnituk (McAllister, 1964). This latter information extends the known range of brook trout northward over 50 miles from the previous northern-most point of Payne Lake reported by Légèndre and Rousseau (1949). Bell (1886) stated that Eskimos captured brook trout in a stream just south of Cape Wolstenholme, but this record lacks confirmation.

Brook trout are found throughout Quebec (Légèndre, 1954) and have been collected as far north as the Ungava region in the George River at Helen Falls, Koksoak River, Lake Aigneau and vicinity, the lower 12 miles of the Larch and Kaniapiskau rivers, the Guignais River, the lower parts of the Whale River (Power, 1966), and the Leaf River (Dunbar and Hildebrand, 1952). On the Labrador coast, brook trout occur along the coast as far north as Nain (Dymond, 1947; Backus, 1957). They also occur naturally in all suitable waters in Newfoundland (Scott and Crossman, 1964), Prince Edward Island (White, 1930), New Brunswick (Scott and Crossman, 1959), and Nova Scotia (Livingstone, 1951).

In the New England States, brook trout are widely distributed (Hubbs and Lagler, 1947) including Cape Cod (Bigelow and Schroeder, 1953). At one time, the range included Long Island, New York (Mitchill, 1815) and extended into the suitable waters of the State of New Jersey (Fowler, 1920). Most of the streams of Pennsylvania were inhabited by brook trout (Fowler, 1940; Miller, personal communication) as well as those streams in the counties of Ashtabula and Geauga in Northeastern Ohio (Trautman, 1957) and the northern and western counties of Maryland (Barry, personal communication).

Native brook trout are still present in headwater streams of the Appalachians in the eastern third of West Virginia (Beall, personal communication) and in approximately 40 western counties of Virginia including the tributaries of the Holston River (Séguin, 1956; Martin, personal communication). In Tennessee, the species inhabits headwater streams of the Tennessee, Holston, and Little Tennessee rivers above an altitude of 900 m (Wilkins, personal communication). It is found naturally in the western part of North Carolina (Cornell, personal communication), in the mountainous northeast corner of South Carolina in Oconee, Pickens, and Greenville counties (Archer, personal communication), and in the headwaters of the Broad, Savannah, Chattahoochee, and Catawba rivers located in the northeast section of Georgia (Jordan et al., 1930; Malone, personal communication).

In Michigan, brook trout were indigenous to waters of the Upper Peninsula, but found in the Lower Peninsula only to the north of a line between Grand Traverse Bay on Lake Michigan and Thunder Bay on Lake Huron (Séguin, 1956). In Minnesota, brook trout were supposedly abundant in the lower reaches of rivers which flow into Lake Superior and in some small lakes above Beaver Bay and Grand Marais (Eddy and Surber, 1947). Brook trout were claimed as native fish in the St. Croix River and the small tributaries of the Mississippi River south of St. Anthony Falls (Eddy and Surber, 1947; Surber, 1931; Carlander, 1954).

The brook trout was found originally throughout Wisconsin (Brasch et al., 1966), and in the Upper Iowa River drainage system in the northeastern part of Iowa (Harlan and Speaker, 1956). O'Donnell (1935) stated that in the past, native brook trout were found in the Kishwaukee, Pecatonica, and Apple river systems of northern Illinois, but were no longer present by that date.

Sea-run brook trout occur within the native range of brook trout. McAllister (1964) observed sea-run fish off the eastern shore of Hudson Bay in the area of Broughton Island. Anadromous brook trout also occur in Ungava Bay (Dunbar and Hildebrand, 1952), on the Labrador coast (Stearns, 1884), and along the coast of Newfoundland (Scott and Crossman, 1964), the Gulf of St. Lawrence, the outer coast of Nova Scotia, the Bay of Fundy, and Prince Edward Island (Smith and Saunders, 1958; Leim and Scott, 1966; White, 1930). Sea-run brook trout or "coasters" also occur in certain rivers along the Maine coast (Bigelow and Schroeder, 1953) and Cape Cod (Bigelow and Schroeder, 1953; Mullan, 1958).

Reductions have occurred in the native range of the brook trout because of changes in the aquatic environment (Fig. 1). In Minnesota, the species is now self-sustaining only in streams of the north-central and northeastern parts (Swenson, personal communication). Brook trout were originally found throughout Wisconsin, but this range has been reduced to include only the east-central and northern portions (Brasch et al., 1966).

Although the brook trout was once native to the waters of northern Illinois (Séguin, 1956; O'Donnell, 1935), Harth (personal communication) states that this species is no longer present since a small put-and-take programme was discontinued in the late 1940's. Native brook trout populations of northeastern Ohio (Kirtland, 1838) had disappeared by 1945 (Trautman, 1957). With dwindling habitat suitable for the brook trout, the Ohio Division of Wildlife terminated its brook trout stocking programme in 1962 (Lakes, personal communication).

Brook trout probably occurred naturally in nearly all of the streams of Pennsylvania, but because of pollution, siltation, and the warming of many waters caused by deforestation (Hazzard (year unknown)) the range of brook trout has been substantially reduced. In New Jersey, where the species was found in nearly all counties, native brook trout populations now exist only in headwater streams of the northwestern counties of Sussex, Warren, Morris, and Passaic (Hayford, personal communication).

NATURALIZED DISTRIBUTION

NORTH AMERICA

United States of America

The distribution of the brook trout has been greatly extended in the United States to include waters of several states west of the Mississippi River (Fig. 1). As early as 1872, Seth Green's trout farm at Caledonia, New York, sent eggs to the California Fish Commission which were distributed in the North Fork of the American River, the headwaters of the Alameda Creek, and the San Andreas Reservoir (Evermann and Bryant, 1919). By 1880, the introduction of brook trout had also been made to Colorado, Utah, Wyoming, and Hawaii. However, as self-sustaining brook trout populations were not established in Hawaii, introductions to that state were discontinued in 1896 (Needham and Welsh, 1953). Ohio, Minnesota, West Virginia, and Michigan, by 1880, were attempting to extend the native range of brook trout within their boundaries by stocking suitable waters.

Attempts to introduce brook trout into 20 states lacking native populations have resulted in their establishment in 14 states (Table 1). Government agencies in these states, excepting Alaska, Nebraska, New Mexico, and North Dakota, continue to stock the species. Among those states from which native populations have declined or disappeared, Iowa, Illinois, and Ohio have discontinued brook trout plantings in favour of the rainbow trout, *Salmo gairdneri* Richardson. The introduction of brook trout has not been attempted in

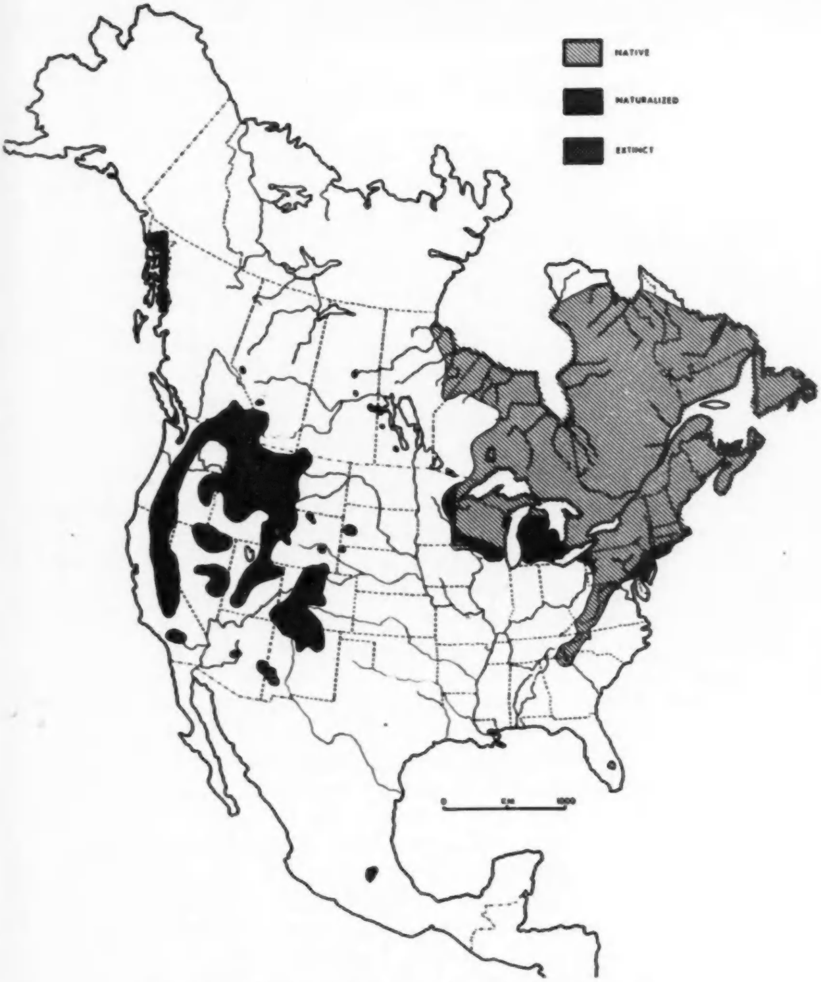

FIG. 1. Distribution of *Salvelinus fontinalis* in North America.

Texas, Oklahoma, Missouri, Mississippi, Florida, Arkansas, Alabama, Louisiana, or Kansas.

CANADA (TABLE 2)

Native or naturalized brook trout populations are resident in all provinces of Canada except the Yukon and Northwest Territories. The transfer, in 1908, of 35,000 ova from Quebec to the Fraser River Hatchery in British Columbia was the first attempt to establish brook trout outside its native

TABLE 1. The history and the present status of the brook trout in the United States of America.

State	Population: native or introduced	Year of introduction	Naturalized distribution	Current stocking programme
Alabama	–	–	–	–
Alaska	Introduced	1920	Yes	No
Arizona	Introduced	Prior to 1930	Yes	Yes
Arkansas	–	–	–	–
California	Introduced	1872	Yes	Yes
Colorado	Introduced	1874	Yes	"
Connecticut	Native	–	–	"
Delaware	Introduced	1950	No	"
Florida	–	–	–	–
Georgia	Both	Unknown	Yes	Yes
Hawaii	Introduced	1876	No	No
Idaho	"	1908	Yes	Yes
Illinois	"	Late 1800's	No	No
Indiana	"	Prior to 1931	No	"
Iowa	Both	1930's	Yes	"
Kansas	–	–	–	–
Kentucky	Introduced	About 1965	No	Yes
Louisiana	–	–	–	–
Maine	Native	–	–	Yes
Maryland	Both	Unknown	Yes	"
Massachusetts	Native	–	–	"
Michigan	Both	1879	Yes	"
Minnesota	Both	1878	Yes	"
Mississippi	–	–	–	–
Missouri	–	–	–	–
Montana	Introduced	1894	Yes	Yes
Nebraska	"	1882	"	No
Nevada	"	1880	"	Yes
New Hampshire	Native	–	–	"
New Jersey	Both	Prior to 1884	Yes	"
New Mexico	Introduced	1892	Yes	No
New York	Native	–	–	Yes
North Carolina	Both	Unknown	Yes	Yes
North Dakota	Introduced	1955	No	No
Ohio	Both	1868	No	No
Oklahoma	–	–	–	–
Oregon	Introduced	Late 1890's	Yes	Yes
Pennsylvania	Both	1916	Yes	"
Rhode Island	Native	–	–	"
South Carolina	Both	Unknown	Yes	"
South Dakota	Introduced	1886	"	"
Tennessee	Both	Unknown	"	"
Texas	–	–	–	–
Utah	Introduced	1875	Yes	Yes
Vermont	Native	–	–	"
Virginia	Both	About 1900	Yes	"
Washington	Introduced	1894	"	"
West Virginia	Both	1878	"	"
Wisconsin	Both	Unknown	"	"
Wyoming	Introduced	1880	"	"

TABLE 2. History and the present status of brook trout in Canada.

Province	Population: native or introduced	Year of introduction	Naturalized distribution	Current stocking programme
Alberta	Introduced	1930's	Yes	Yes
British Columbia	Introduced	1908	"	"
Manitoba	Both	1942	"	"
New Brunswick	Native	–	–	"
Newfoundland	Native	–	–	"
Northwest Territories	–	–	–	–
Nova Scotia	Native	–	–	Yes
Ontario	Native	–	–	"
Prince Edward Island	Native	–	–	"
Saskatchewan	Introduced	Late 1920's	Yes	"
Yukon Territory	–	–	–	–

range (Department of Marine and Fisheries, 1908; Clemens and Wilby, 1961). The subsequent distribution of fry led to naturalized populations in several waters in the southeastern part of the province and on Vancouver Island (Clemens and Wilby, 1961; Carl et al., 1959). An active brook trout stocking programme is still maintained by the British Columbia Department of Recreation and Conservation (Terpenning, personal communication).

Brook trout were widely introduced to Alberta sometime during the 1930's, and became established in the Highwood and Elbow rivers as well as in high altitude tributaries (1500 m elevation) of the Red Deer and North Saskatchewan rivers in the National Parks at Banff and Jasper (Miller, 1950). The Department of Lands and Forests of Alberta now stock only relatively small numbers of brook trout each year in suitable waters (Paetz, personal communication).

In Saskatchewan, brook trout have been planted in some 20 streams of the Cypress Hills (1200 m elevation) in the southwestern part of the province between 1931 and 1958. Those in which brook trout have become naturalized include Bear, Belanger, Little Frenchman, Battle, Bone, and Loch Lomond creeks (Symington, 1959; Marshall, personal communication). In the Mossy River drainage of Nipawin Provincial Park, nine streams have been stocked since 1934 but only three, Lost Echo, McDougal, and White Gull, have known naturalized populations of brook trout. Marshall (personal communication) states that although the present naturalized distribution does not include La Ronge nor the lower Red Deer River, self-sustaining stocks of brook trout are found in four rivers of the Pasquia Hills (elevation 750 m) namely the Otosquen, Pasquia, Prairie, and Fir rivers. The Department of Natural Resources still continues an active brook trout stocking programme (Johnson, personal communication).

Brook trout have been stocked in Manitoba since 1942, when ova were obtained from hatcheries at Dorion, Ontario, and Spokane, Washington

(Benedictson, personal communication). Naturalized populations have been established in the Birch and Steeprock rivers of the Porcupine Mountain Forest Reserve, in streams of the Duck Mountain Forest Reserve, as well as Stoney Creek near Neepawa (Benedictson, personal communication; Keleher and Kooyman, 1957).

MEXICO

Although the breeding and propagation of brook trout in Mexico was considered as early as 1937 by Berriozabal (1937), the species was not introduced until 1945 when streams near El Zarco, State of Mexico, were stocked (Mexico, Department of Fisheries, personal communication). Chávez (personal communication) states that brook trout reproduce naturally in those streams at elevations near 3000 m, although certain lakes and streams must be replenished periodically with hatchery-reared stocks. Apparently, the brook trout is considered of minimum importance and more emphasis has been placed on stocking programmes utilizing the rainbow trout, *S. gairdneri*.

EUROPE

(Table 3)

THE BRITISH ISLES

The first introduction of brook trout to the British Isles occurred in the spring of 1869, when Livingstone Stone of the Cold Spring Trout Hatchery in New Hampshire, USA, sent a consignment of ova to Frank Buckland, Her Brittanic Majesty's Commissioner for Fisheries; a second shipment of 10,000 eggs was sent in 1871 to John Parnaby, owner of the Troutdale Fishery near Keswick (de Bunsen, unpublished data). Today, the only hatchery in the United Kingdom which rears brook trout commercially is the Chirk Fisheries Station, Chirk, near Wrexham, North Wales (Campbell, personal communication).

Although brook trout have been extensively introduced to many waters of the British Isles, such as the lochs in the estate of Maclaine of Lochbuie, Island of Mull, Scotland (MacLean, 1923), the species has become firmly established in only a few localities (de Bunsen, unpublished data) (Fig. 2). Naturalized populations exist in England in the County of Surrey at Haslemere on the Surrey Trout Farm, and in the ponds of Sir Hildebrand Harmsworth. In Scotland, a population has been established in a small loch at Ochtertyre Estates near Crieff, Perthshire. In Wales, a naturalized population exists at Llyn-y-Tarur, near New Town, Montgomeryshire. Stocks of brook trout which are either precarious, or recently introduced, are found in the Fisheries Biological Association tarns on Claife Heights near Lake Windermere and the Chew Reservoirs, Bristol, England. In Scotland, recently stocked brook trout are present in a pond near Tayfield, Fife, and at Fincastle Loch in Perthshire; they have never been introduced into Ireland (de Bunsen, unpublished data).

TABLE 3. Introductions and present status of the brook trout in Europe.

Country	Year of introduction: First	Year of introduction: Successful	Source	Survival	Current stocking programme	Naturalized distribution
Austria	After 1879	After 1879	Germany	Yes	Yes	Yes
British Isles	1869	1869	USA	"	No	Yes
Bulgaria	1930	1930	Czechoslovakia	"	Yes	Unknown
Czechoslovakia	Prior to 1890	Prior to 1890	Unknown	"	Yes	Yes
Denmark	About 1900	About 1900	Germany	"	No	"
Fed. Rep. Germany	1879	1879	USA	"	"	"
Finland	1898	1967	USA & Denmark	Unknown	"	No
France	1883 approx	1883	USA	Yes	"	Yes
German Dem. Rep.	1879	1879	USA	Yes	Yes	Yes
Hungary	Before W.W.II	none	Germany	No	No	No
Italy	1891	After W.W.II	Denmark	Yes	"	Yes
Netherlands	Prior to 1883	None	Unknown	No	"	No
Norway	1870	1918	Unknown	Yes	"	Yes
Poland	After 1879	After 1879	Germany	"	Yes	"
Roumania	Before 1900	Before 1900	Austria	"	"	"
Spain	1934	After W.W.II	France	"	"	Unknown
Sweden	1891	1891	Germany	"	"	Yes
Switzerland	1883	1883	Germany	"	"	Yes
USSR	Prior to 1914	After 1930	Unknown	"	Unknown	No

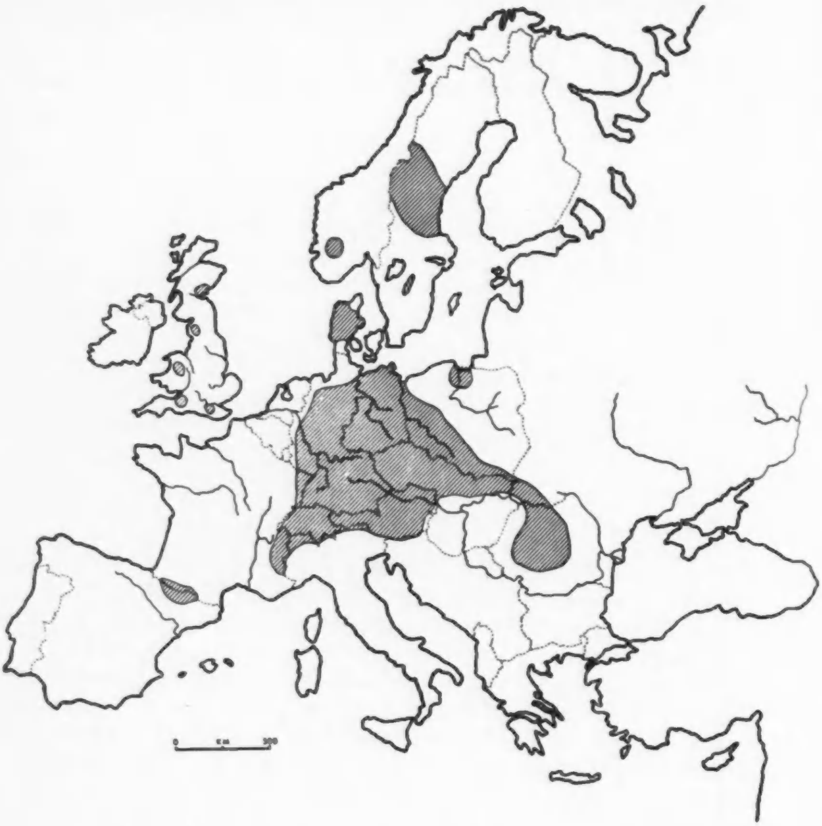

FIG. 2. Naturalized distribution of *Salvelinus fontinalis* in Europe.

AUSTRIA

Brook trout, probably of German origin, were introduced into Austria shortly after 1879. The species has now been naturalized in nearly all of the Austrian provinces including Vorarlberg, Tyrol, Salzburg, Upper Austria, Lower Austria, Styria, and Carynthia (Kähsbauer, personal communication).

BULGARIA

The first attempt to introduce brook trout into Bulgaria was made in 1930, when eyed ova were imported from Czechoslovakia and hatched in government ponds near the town of Samokov in western Bulgaria (Zashev, personal communication). This introduction was a failure but, in 1959, another attempt was made to populate several closed mountain lakes with brook trout, care being taken to avoid hybridization with brown trout (Zashev, personal communication). Over 1 million brook trout fry are produced each year by state hatcheries and, since 1962, some have been planted in a mountain lake at 1500 m altitude. Although 4-year-old fish, averaging 600–700 g and individuals weighing up to 1500 g are caught, Zashev states that it is too early to consider brook trout naturalized in Bulgaria.

CZECHOSLOVAKIA

Brook trout were first stocked prior to 1890 in Black Lake of the Šumava Mountains in southwestern Bohemia (Lohinský, personal communication). Fish are now planted mostly in valley reservoirs of northern Bohemia and suitable streams and brooks throughout the country. Naturalized populations have developed in the High Tatra Mountains of northern Slovakia, in middle and eastern Slovakia, in the Hrubý-Jeseník Mountains of northern Moravia, in the Bohemian-Moravian Highland, and in the Krkonoše and Jizerské mountains of northern Bohemia (Lohinský, personal communication). Lohinský states that brook trout show the best rate of growth in Lake Štrbské Pleso in the High Tatra Mountains, where specimens have attained 500 mm in total length and 1000 g in weight.

DENMARK

Brook trout are presumed to have been first introduced into trout farms of central Jutland from Germany about 1900 (Larsen, personal communication). Naturalized populations have developed downstream from several trout farms after the escape of individuals. Successful plantings of this species have recently been made in the River Skjern å and the Randers fjord and other populations are found in cool tributaries of the River Lindenborg å and Hald Lake. In 1966, two specimens were caught in the Ringkøbing fjord, one specimen in spawning dress (Larsen, personal communication).

FINLAND

Importations of brook trout ova to Finland were made in 1898, 1901, 1904, 1906, and 1912 from Germany to the Evo Research Station (Toivonen, personal communication, 1967). There is no evidence that plantings of fry and fingerlings of this early stock made in small lakes and rivers of the area established naturalized populations.

Planting experiments with yearling trout from the USA and 2-year-old fish from Denmark, begun in 1967, are too recent for an evaluation of success (Toivonen, personal communication).

FRANCE

Importation of brook trout (saumon de fontaine) ova from the United States to France first occurred about 1883 (Anonymous, 1884). Although brook trout were liberally planted, no naturalized populations have become established in lentic waters such as the upper Seine River, the tributaries of the Rupt-de-Mad in Lorraine, or in the streams of the Alps or the Vosges

(Vivier, 1955). However, brook trout are naturalized in high altitude mountain lakes of the Alps within the province of Dauphiné (Lac Lutel, Domenon, and Prénol), and in other lakes and streams at elevations between 1800 m and 2300 m in the Alps within the province of Savoie, where brown trout are not present. Naturalized populations of brook trout are established in the Pyrenees Mountains in the upper drainage basins of the Aspe and Gaube streams at elevations of approximately 2400 m, and in ponds at Sturzelbronn and Mouterhouse near Bitche (Moselle) (Vivier, personal communication).

GERMANY

First introductions of brook trout into Germany were made in 1879. Livingstone Stone sent a quantity of eggs to von Behr of the German Fishery Association (von Behr, 1883). In 1885, the Association sent brook trout ova to a hatchery at Boitzenburg, Mecklenburg which is now situated in the German Democratic Republic (East Germany) (Dem Borne, 1885). Naturalized populations of brook trout are found throughout the Republic in small streams, but they are more numerous in Thüringen and the Werra River and its tributaries (Steffens, personal communication). Several hatcheries produce brook trout for stocking small ponds and streams.

Naturalized populations of brook trout are now present throughout the Federal Republic of Germany (West Germany) in brooks, cold fast-running streams, and mountain lakes (Liebmann and Reichenbach-Klinke, personal communication, 1967). Though the Federal Government does not stock brook trout, several fisheries associations carry out private lake plantings. Liebmann and Reichenbach-Klinke state that, in some waters, brook trout cross with brown trout (*Salmo trutta* L.) and give rise to the sterile hybrid (Tigerfisch).

HUNGARY

Széky (personal communication) states that, although brook trout were introduced privately before World War II, the species is not known to exist in Hungarian waters at the present time.

ITALY

The first attempt to introduce brook trout to Italy was made in 1891 when fry were planted in Lake Idro, Lombardia, North Italy. This early introduction, as well as one made in 1935, to streams of the Valley of Aosta (Piedmont) were failures (Tortonese, personal communication). After World War II, the Fishery Council of the Aosta Valley obtained brook trout ova from Denmark and placed them in a local hatchery. Fry were planted with some success in this northern section of Italy, and naturalized populations have become established in at least two lakes of the Aosta Valley (elevations of 1940 m and 2542 m) where competition from other salmonids does not occur (Tortonese, personal communication).

THE NETHERLANDS

Brook trout eggs were imported on two occasions prior to 1883 (Anonymous, 1884) but both of these attempts were failures. The species is not present in the waters of the Netherlands although rainbow trout, *S. gairdneri*, have been successfully introduced and are being propagated for angling purposes (de Groot, personal communication).

NORWAY

Between the years 1870 and 1919, brook trout (bekkerøye) were introduced to Norwegian waters at several locations but, as far as is known, no naturalized populations have resulted (Rosseland, personal communication). However, 1000 brook trout fry released in 1918 in a small tarn at Dyrdal, Øyfjell, in the Telemark district have given rise to self-sustaining populations in local creeks and rivers at about 600–1000 m above sea level (Grande, 1964). In the downriver sections of the naturalized brook trout distribution, Grande (1964) found this species co-existing with brown trout.

POLAND

Although the exact date and location of the first introduction to Poland is not known, brook trout eggs were imported from Germany in the last decade of the 19th century (Sakowicz, personal communication). A very limited stocking programme exists utilizing offspring from brood stocks kept at the Trout Pond Farm of the Polish Anglers Society in Opole voivodship, and in the River Laboratory of the Inland Water Fisheries at Gdańsk-Oliwa. Naturalized populations of brook trout exist in a few mountain lakes and rivers, most notably a tributary of the Warta River near Biedrusko, and a small stream near Jelenia Góra, Wroclaw voivodship in western and northwestern Poland. However, the high vulnerability of this species to angling pressure and natural hybridizing with brown trout, *S. trutta*, limit its numbers (Sakowicz, personal communication).

ROUMANIA

According to Banarescu (personal communication) brook trout of Austrian origin were stocked in tributaries of the Somesul Mic River of Cluj county (i.e., Negrutza, Dumitreasa, and Irisoara rivers) near the end of the 19th century. Fish ponds for artificial breeding of salmonids existed in the village of Racatau, near the Negrutza River, until World War I. Naturalized reproducing populations are still found in the Negrutza River and the following waters: a tributary of Crisul Repede R. in the Bihor Mountains (Western Carpathians), Gudea Mica rivulet (a tributary of the Upper Mures River in Transylvania), the Putna rivulet (tributary of the Moldova River in Moldavia), and in the Upper Bistritza River within Moldavia (Banarescu, personal communication).

SPAIN

Brook trout were first imported to Spain from Switzerland in 1934, when ova were placed in the hatchery of the Monastery of Piedra (Elegido, personal communication). These fish, however, were lost during the Spanish Civil War of 1936 to 1939. After World War II, fry were obtained from France and placed in streams of the Pyrenees. However, it is not known if these plantings have resulted in the establishment of naturalized populations. Fish hatcheries in Spain are currently engaged actively in the culture of brook and rainbow trout (Elegido, personal communication).

SWEDEN

Fertilized brook trout ova (bäckröding) were introduced from Germany during the winter of 1891–92 to the province of Jämtland (Svardson, 1964). During the following 15 years, the species was planted in about 120 different waters, most of them in Jämtland, but some also in the provinces of Halsingland and North Dalarna (Svardson, 1964). Naturalized populations have resulted. Alm (1920) states that brook trout have succeeded in certain rivers, lakes, and tarns of the provinces of Örebro, Kopparberg, and Jämtland. Self-sustaining stocks of brook trout occur, also, in small tarns and brooks of the province of Norrland (Hanström and Johnels, 1962; Montén, personal communication).

SWITZERLAND

In 1883, a number of brook trout eggs were sent to Switzerland by the German Fishery Association and hatched in the Roveray Fish Cultural station situated near Allaman (Goll, 1887). In 1885–86, Fred Mather of Cold Spring Harbor, New Hampshire, USA, sent 10,000 brook trout ova to the Government of Switzerland (Clark, 1887). Although naturalized populations of brook trout do exist in Switzerland, intensive stocking of both native and introduced salmonids is required because of the loss of habitat suitable for reproduction (Swiss Fisheries Department, personal communication).

UNION OF SOVIET SOCIALIST REPUBLICS

Brook trout were introduced to the USSR prior to 1914, when 700 fry were stocked in two small lakes and a river in the province of Pontoaralokaspian (Werygin, personal communication). These plantings failed, but in the 1930's the rearing of brook trout was initiated in certain pond-fish farms of the USSR including the "Ropsha" farm near Leningrad, two fish farms in the West Ukraine and Lithuania, as well as the ponds of the Institute of Fisheries and Fish Industry (GOSNIORH) at Leningrad (Savvateeva, personal communication) Werygin states that self-sustaining populations of brook trout have not been recorded from the natural waters of the USSR.

ASIA

INDIA

Brook trout were introduced to India when, in 1963, eyed ova from the United States were placed in trout ponds of the Harwan Trout Farm in the State of Kashmir (Tripathi, personal communication). This stock was successfully bred in 1966 and brood stock was made available to other trout farms of the Kashmir Valley (Tripathi, personal communication).

JAPAN

Although attempts were made to introduce salmonids to Japan prior to 1900, the first successful introduction of brook trout occurred in 1901 when eggs were transported from the United States to the Nikko Hatchery and the resulting fry were planted in the Yugawa River, Nikko City (Shiraishi, personal communication). This species has become naturalized in Kotoku Numa, Yuniko Lake, Yugawa River all located near Nikko City, in Tochigi Prefecture, and in Myozinike Pond, Nagano Prefecture (Shiraishi, personal communication.)

SOUTH AMERICA

ARGENTINA

The first introduction of brook trout (trucha de arroyo) to South America occurred in 1904 when ova were shipped from the United States to a hatchery in Argentina on the shores of Lago Nahuel Huapí (Thompson, 1939). Between the years 1904 and 1931 over 587,000 brook trout eggs were imported and the resulting fry and fingerlings were planted in lakes of Patagonia and Nahuel Nuapí National Park (Thompson, 1939). This species, as well as brown and rainbow trout, have become naturalized in nearly all suitable rivers and large lakes (General Paz, Fontana, Cardiel lakes) of the Patagonian Steppe (Mastrarrigo, personal communication) (Fig. 3). It is stated by de Plaza and Plaza (1949) that brook trout are abundant in the National Parks of Nahuel Huapí, Lanín, and Los Alerces, and according to Thompson (1939), brook trout are, in fact, the most numerous salmonid in Nahuel Nuapí.

BOLIVIA

Brook trout were introduced from Chile in 1948 and placed in the Pongo Hatchery (Terrazas, personal communication). Several rivers of the Cordillera Occidental and Altiplano were stocked and now, even though no regular stocking programme exists, self-sustaining populations have developed (Terrazas, personal communication).

CHILE

The introduction of brook trout ova to Chile first occurred in 1935–36 but neither the source of stock nor the places of dissemination are known (Astete, personal communication). The culture and stocking of salmonids

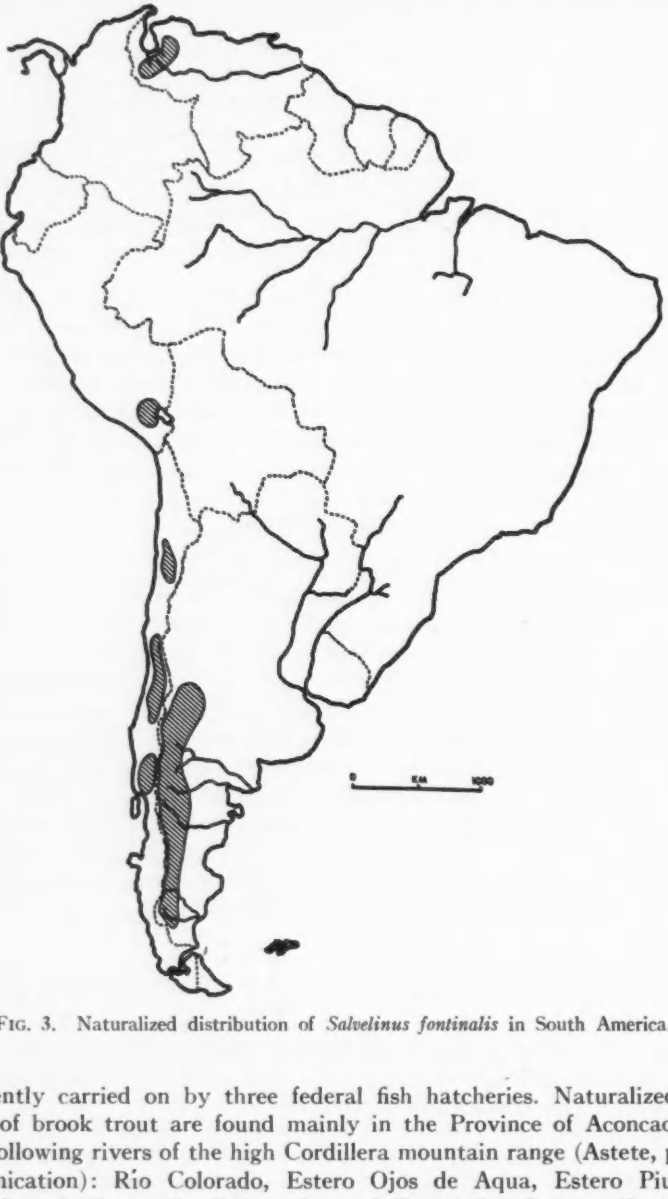

FIG. 3. Naturalized distribution of *Salvelinus fontinalis* in South America.

is presently carried on by three federal fish hatcheries. Naturalized populations of brook trout are found mainly in the Province of Aconcaqua and in the following rivers of the high Cordillera mountain range (Astete, personal communication): Río Colorado, Estero Ojos de Aqua, Estero Piuquenes, Estero Juncal, Estero La Polvareda, and Estero Los Leones. Brook trout sport fisheries occur in the Negro River near Peulla, the Petrohue River flowing out of Todos los Santos Lake, and the Manso and Puelo rivers which flow from

Argentina (Heusser, 1964). Heusser (1964) states that some brook trout, as well as brown and rainbow trout, exist in Laguna de Inca near Portillo (elevation of 2700 m).

COLOMBIA

Brook trout were introduced to Colombia on a very small scale in 1955 when fry from the United States were planted in the Río Teusacá, a slow-moving but cold-water creek, 15 miles from Bogotá (Gómez, personal communication). Some specimens ultimately were caught but it now seems that the species has disappeared from this river (Gómez, personal communication).

ECUADOR

Though the source and date is not known, one attempt to introduce brook trout to Equador was made when fry were planted in the Río Toachi (Mino, personal communication). Although this introduction was a failure, the Department of Industry and Commerce is very interested in making further attempts to naturalize this species.

PERU

Salmonid species were first introduced to Peru in 1928 (Tovar S., personal communication). Brown and brook trout are held currently in Fish Culture Stations at Chucuito and Huaraz, State of Puno, near Lake Titicaca where both species have become naturalized in lakes and rivers above an elevation of 2500 m above sea level (Tovar S., personal communication). The culture of brown, rainbow, and brook trout is conducted also, in the states of Junín, Santa Eulalia, Cajamarca, Huaraz, and Huánuco encompassing elevated regions of the Andes (Tovar S., personal communication).

VENEZUELA

Weibezahn (personal communication) reports that brook trout were first introduced to Venezuela in 1937 from the USA. These introductions continued until 1942 with plantings in the lakes and rivers of the Andes/Venez. The species is stocked sporadically and is now naturalized in the Motatan, Chama, and Santo Domingo rivers in the State of Mérida, and in many small lakes in the Paramos of the Venezuelan Andes.

FALKLAND ISLANDS

The introduction of trout to the Falkland Islands would seem to have been attempted during World War II when small quantities of eyed ova of brown, rainbow, and brook trout were brought from Chile (Arrowsmith and Pentelow, 1965). Brook trout are resident in one stream, the Moody Brook (Arrowsmith and Pentelow, 1965).

AFRICA

SOUTH AFRICA

Brook trout ova were imported from the USA to South Africa by air in 1950 and placed in a commercial trout hatchery in the Cape Province where a breeding stock was established (Smith, personal communication). Progeny were distributed to all suitable waters in the Cape Province including Steenbras Reservoir and the upper Witte River. However, no wild populations were established (Smith, personal communication).

A further importation of eggs from the USA was made in 1961 to establish brood stock at the Jonkershoek Hatchery but ultimate attempts to naturalize this species in local waters was again a failure. Male brook trout are now kept at the Jonkershoek Hatchery solely for crossing with the brown trout (*S. trutta*) to produce the "Tiger Trout" hybrid (Smith, personal communication).

The commercial hatchery in the Transvaal did not maintain its stock of brook trout for long as rainbow and brown trout were considered of greater importance (Smith, personal communication).

KENYA

A small consignment of brook trout ova were imported from England in 1949, but the stocking of waters at high altitude (2000 m) with fry subsequently hatched from these ova was a failure (Harrison et al., 1963). Again in 1961, ova were imported from the Paradise Brook Trout Co. of Pennsylvania, USA, and the fingerlings were stocked in a large high altitude reservoir in the Aberdare Mountains and a few rivers of this area (Watson, personal communication). Very few survivors have been seen in the reservoir but the species is known to have bred successfully in one of the rivers (Watson, personal communication).

FEDERATION OF RHODESIA AND NYASALAND

The first attempt to naturalize brook trout in this area was made in 1908 when ova were imported from England and the fry placed in the Mlungusi River (Harrison et al., 1963). This attempt, and a 1955 planting of streams in the Inyanga area were failures (Harrison, 1963).

TANZANIA (TANGANYIKA)

In 1953 and 1954, brook trout ova were obtained from the Jonkershoek Hatchery in South Africa and stocked in the Ndumbi and Rumakali rivers on the Elton Plateau (elevation of 2600 m) (Harrison et al., 1963). However, this experiment failed so, in 1958, these two waters were stocked with rainbow trout, *S. gairdneri* Richardson (Harrison et al., 1963).

AUSTRALIA AND NEW ZEALAND

AUSTRALIA

Brook trout were introduced to mainland Australia, and by 1908 had succeeded in adapting themselves to certain waters, particularly those of the Snowny River district (Stead, 1906). Roughley (1951) and Weatherly and Lake (1967) make no mention of this species, and so it is assumed that brook trout are now absent, although brown and rainbow trout exist as self-sustaining populations on the mainland.

Although brook trout were introduced before 1907 to lakes Leake, Crescent, and Sorell in Tasmania, naturalized populations did not result (Lynch, personal communication). In 1962, 50,000 brook trout ova were received from the hatchery at Collingwood, Nova Scotia, Canada, and the progeny of these ova are still being artificially propagated at the Salmon Ponds in Tasmania. Liberations have been made since 1963 in suitable ponds, lagoons, and lakes of the State but, as of February 1967, there was no evidence to indicate that self-supporting stocks had been established in Tasmania (Lynch, personal communication).

NEW ZEALAND

The earliest planting of brook trout in New Zealand was made in 1877 near Auckland by an "Acclimatization Society" but this, and further attempts to naturalize the species in 1880 and 1881 were unsuccessful (Little, personal communication).

However, in 1890, the Horokoro in Rotorua was stocked with brook trout. This planting, as well as those made in the upper reaches of a few North Island streams and Lake Emily in mid South Island, have led to naturalized reproducing populations of brook trout (Little, personal communication).

DISCUSSION

Naturalized populations of brook trout have been established beyond its native range by introduction into the waters of 4 provinces and 14 states in North America and 19 countries in Eurasia, Central and South America, as well as New Zealand. The present discontinuity in distribution, whether considered on a local, continental, or world basis, may be considered to reflect the sensitivity of the species to environmental conditions rather than to represent a lack of attempts to establish the species more widely. The species does not, however, always enjoy the prestige in all parts of the world that it does within its native range.

An analysis of the pattern of world distribution suggests a positive relationship between the presence of self-sustaining populations and those environmental factors which affect water temperature, namely latitude, altitude, and precipitation. In general, plantings of brook trout have resulted in naturalized populations only in those areas offering, as well, geophysical and ecological conditions similar to those occurring within the native range.

A review of the literature reporting on laboratory and field investigations on the environmental requirements of salmonids reveals a general agreement that water temperature is the most important single factor limiting geographic distribution (Baldwin, 1951; Vladykov, 1963). The thermal range of water temperature tolerated by the brook trout under laboratory conditions extends from 0 C upwards to an ultimate upper incipient temperature of 25.3 C, or to 28.5 C for short intervals, depending on acclimation (Fry, 1947, MS, 1951). Observations made on natural populations confirm the prolonged existence of the species in water ranging from near freezing to about 24 C (Ricker, 1934; La Rivers, 1962).

Brook trout are not found regularly in lotic or lentic areas with temperatures much above 20 C if a lower temperature is available to them (Henderson, 1963), although trout in lakes may make temporary excursions from deeper and colder water into the warmer epilimnion during the period of summer stratification (Fry, MS, 1951). Brook trout, when placed in a thermal gradient in the laboratory select a temperature of 14–16 C (Graham, MS, 1948; Fisher and Elson, 1950). Observations by the authors tend to confirm that this preference occurs in stratified lakes but may not occur in streams where population density and habitat conditions may be more dominant in establishing the distribution of fish. It is of interest that the most suitable temperature for the general performance of the species would seem to lie in the range of the preferred temperature.

Davis (1961) states that temperatures of 11–14 C are most satisfactory for rapid growth of fingerlings and Baldwin (1951) cites an optimum growth rate for brook trout at 14 C. Fisher and Elson (1950) point out that the optimum temperature for the greatest response to an electrical stimulus coincides with the selected (or preferred) temperature. Graham (1949) found the cruising speed of brook trout to be greatest at 20 C, but at 16 C the species had the most efficient oxygen uptake for physical activity. Fisher and Sullivan (1958) calculated the spontaneous activity of brook trout to be at a maximum at that temperature selected in a temperature gradient.

It would seem, therefore, that although brook trout may survive over a wide range of sublethal temperatures, water in the 11–16 C range is best suited to their overall welfare, and at both higher and lower temperatures the fish exist at a relative disadvantage in terms of activity and growth (Baldwin, 1951). Although the effects of low temperature in nature have received little attention, brook trout within their native range can be self-sustaining in cold headwater streams and ponds which rarely, if ever, exceed 10 C, but with greatly inhibited growth.

Although brook trout may survive to maturity over a wide range of temperatures, water temperature becomes more critical at the time of spawning and egg incubation when, for successful natural reproduction, it must fall within a rather narrow range. Under natural conditions in Ontario, the brook trout spawns normally at about 9 C, the temperature of ground water where the gravel redds are located. Embody (1934) observed that brook trout eggs fail to develop at temperatures above 13 C and suggested about 11.5 C as the safe upper limit. He observed, also, that a temperature above 4.5 C was necessary during incubation to the eyed stage but that egg development can be completed at temperatures as low as 1.7 C but with abnormally high losses and likely less robust fry. Incubation time is a function of water temperature and, within the temperature range considered suitable for egg incubation (4.5–11.5 C), the time from fertilization to hatch varies between about 7 and 13 weeks (Davis, 1961).

Evidence of the sensitivity of the brook trout to environmental influences is provided by observations on the species within its native range. In some areas south of the Pre-Cambrian Shield, where land-use patterns accompanying 200 years of settlement have caused marked ecological change, local populations have been eliminated. More commonly, in the southern Great Lakes drainage and southward, the local stream distribution of the species has become limited to headwaters because of loss of pristine habitat through erosion, siltation, and seasonally high water temperatures.

The native range of brook trout is delimited to the north both by the Arctic Ocean and a mean daily air temperature of −15 C in January (Department of Mines and Technical Surveys, 1957) and to the south by the isotherm marking a mean July air temperature not exceeding 21 C (Séguin, 1956). The area of distribution extends from the Atlantic Ocean westward to the line of 64 cm average annual precipitation (Visher, 1954) or to the place where the annual surplus precipitation becomes less than 10 cm (Séguin, 1956). Plantings of brook trout in the Central Plains of North America have generally led to the establishment of self-sustaining populations in proximity to sources of headwater streams where summer water temperatures remain tolerable. It would seem, therefore, that areas characterized by mixed deciduous or coniferous forests, or both, a cold temperate climate, cool spring-fed surface waters, and moderate precipitation are suited for the establishment of self-sustaining brook trout populations.

Naturalized populations of the species have been established by introduction only in those areas of the world where meteorological conditions are remarkably similar to those within its native range. Extension of the range in North America has proven possible only in elevated areas (at least 600 m above sea level) or where annual rainfall amounts to more than 42 cm, or both. Similarly in Europe, plantings have been the most successful in elevated areas of the Pyrenees and Alps, and of the Scandinavian and Carpathian mountains. The European distribution is delimited by a mean daily July air temperature of 24 C and an annual rainfall of 50 cm. In France, the species seems to adapt best to high mountain lakes and headwater streams where competition from other salmonids is least (Vivier, 1955). In South America, also, brook trout have been established in headwater streams of the Andes as well as inland lakes of the Patagonian Steppe. However, brook trout populations in Africa, Australia, and New Zealand appear unable to compete with the brown or rainbow trout, or both, and so have been largely unsuccessful.

In some instances, the introductions of brook trout beyond the native range have resulted in the establishment of naturalized populations in cold and relatively sterile environments often favourable for survival and reproduction but not necessarily conducive to good growth. For, example, in certain high altitude lakes of California, a combination of overpopulation, cold water temperature, and inadequate food contribute to slow growth and small fish (McAfee, 1966). Similar biological features are typical of trout introduced into high altitude or headwater streams on all continents and, consequently, the species may lack the prestige which it enjoys as a sport fish within its native range. However, in some areas of the world, such as Peru, Argentina, Venezuela, and Japan, naturalized populations of brook trout have been very successful in terms of growth and angling quality.

In conclusion, it may be stated that the brook trout, in spite of numerous unsuccessful efforts to establish it, is now widely naturalized in North America beyond its native range and on the continents of Eurasia and South America. The species, however, has not gained the respect as a food or sport fish afforded native salmonids or, where introduced, the exotic rainbow trout. Waters suited for the survival, reproduction, and favourable growth of brook trout are very limited beyond its native range because of the species low tolerance to adverse environmental conditions. Attempts to introduce it into habitats occupied by other salmonids have had only marginal success and, in cold environments, individuals show relatively slow growth and poor condition.

Because of the foregoing factors, it is improbable that, with the possible exception of Asia, the present range of the brook trout will be extended greatly through further attempts at introduction.

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A Synopsis and Key to the Recent Ophiuroidea of Washington State and Southern British Columbia

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KYTE, MICHAEL A. 1969. A synopsis and key to the recent Ophiuroidea of Washington State and southern British Columbia. J. Fish. Res. Bd. Canada 26: 1727–1741.

Twenty-four species are listed with synonomies according to the classification of Spencer and Wright (Treatise on invertebrate paleontology, p. U78–U107, Vol. 1, 1966). Geographical and bathymetrical distributions are also listed. The feeding habits and substrate preferences are listed wherever possible and are correlated with the distributions. A key to the 24 species is presented.

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INTRODUCTION

ALTHOUGH THE OPHIUROIDS are highly important ecologically in many marine benthic communities, they have been relatively neglected in the ecological literature. Many workers and expeditions have collected and reported ophiuroids from Washington and British Columbia. Yet, until recently, there has been little attention paid to the species and their distribution in this area. However, due to recent increased interest in marine benthic communities, there appears to be a definite need for a checklist, key, and summary of the distribution of ophiuroids in Washington and British Columbia.

This paper gives such a checklist and key, with synonomies, of the species found in Washington and southern British Columbia. In conjunction, a tentative distribution of ophiuroids in these areas is proposed with reference to physical and biological factors. The ophiuroid populations of other areas are being studied at present and the results will be published when completed.

SYNOPSIS AND KEY TO THE WASHINGTON AND SOUTHERN BRITISH COLUMBIA OPHIUROIDEA

Since 1915 the systematics of ophiuroid echinoderms has undergone several whole and partial revisions and is still quite unsettled. In 1915, Matsumoto proposed a new classification based on general characters of the skeleton. This classification abandoned the traditional use of "Euryale" and "Ophiurae" as principal divisions. A few authors, H. L. Clark, R. Koehler, and S. Murakami (Fell, 1962b), accepted this classification immediately.

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On the other hand, H. B. Fell, Th. Mortenson, A. M. D'jakonov, and several other writers rejected it for various reasons (Fell, 1960, 1962b).

However, Dr H. B. Fell published in 1962 new evidence supporting Matsumoto's original classification. In a short paper Fell elaborated on this evidence and proposed a new classification on the basis of the knowledge accumulated since Matsumoto (1915, 1917) and on the new findings (Fell, 1962b, 1963a). More new evidence (Fell, 1963b) supported a further revision, this time of the entire phylum. This revision placed the Asteroidea and Ophiuroidea along with several fossil groups into the subphylum Asterozoa as subclasses. The classification has subsequently been used by several authors. The present paper uses a further modified classification as set out by Spencer and Wright (1966).

Two partial revisions of the ophiuroids also affected the classification as used in this paper. The first is Fell's (1962a) revision of the family Amphiuridae creating several new genera and redefining others. The second is Thomas' (1966) revisions of the genus *Amphipholis* placing *A. squamata* and possibly *A. pugetana* in the new genus, *Axiognathus*. In the present paper I have placed *Amphipholis pugetana* in the genus *Axiognathus* because of its extreme similarity to *A. squamata*. Both of these revisions are used throughout the paper.

Although there is much taxonomic literature dealing with ophiuroids, only a few reliable keys are available. A few regional keys are available in the literature, published and unpublished. One of these regional keys is the Boolootian and Leighton (1966) key to the ophiuroids of the southern California areas. One other key to Californian ophiuroids may be found in Light's Manual (Light et al., 1961). Fell has published two keys that are highly useful: one (Fell, 1960) is a synoptic generic key; the other (Fell, 1962a) is a key to the Amphiuridae genera which is included in his revision of this large family.

The 24 species of ophiuroids reported from Washington and southern British Columbia are keyed in this paper. The terms used in the key and the paper in general are those terms that are most commonly used in the recent literature. For illustration of systematically significant structures Fell (1960) should be consulted.

These species are listed below with synonomies and their distributions. The family arrangement follows that of Matsumoto (1915, 1917), Fell (1962a, b) and Spencer and Wright (1966). Classification of bathymetrical zones follows Hedgpeth (1957).

PHYLUM Echinodermata Klein, 1734
SUBPHYLUM Asterozoa Zittel, 1895
CLASS Stelleroidea Lamarck, 1816
SUBCLASS Ophiuroidea Gray, 1840
ORDER Phrynophiurida Matsumoto, 1915
SUBORDER Ophiomyxina Fell, 1962b
Family OPHIOMYXIDAE Ljungman, 1866

TABLE 1. List of Ophiuroidea from Washington and southern British Columbia.

- PHYLUM Echinodermata
  - SUBPHYLUM Asterozoa
    - CLASS Stelleroidea
      - SUBCLASS Ophiuroidea
        - ORDER Phrynophiurida
          - SUBORDER Ophiomyxina
            - Family OPHIOMYXIDAE
              - *Ophioscolex corynetes*

          - SUBORDER Euryalina
            - Family ASTERONYCHIDAE
              - *Asteronyx loveni*

            - Family GORGONOCEPHALIDAE
              - *Gorgonocephalus caryi*

        - ORDER Ophiurida
          - SUBORDER Chilophiurina
            - Family OPHIURIDAE
              - *Amphiophiura superba*
              - *Ophiocten pacificum*
              - *Ophiura cryptolepis*
              - *Ophiura leptoctenia*
              - *Ophiura lütkeni*
              - *Ophiura sarsii*
              - *Ophiomusium lymani*
              - *Ophiomusium multispinum*

          - SUBORDER Laemophiurina
            - Family OPHIOCANTHIDAE
              - *Ophiolimna bairdi*
              - *Ophiophthalmus normani*

          - SUBORDER Gnathophiurina
            - Family OPHIACTIDAE
              - *Ophiopholis aculeata*
              - *Ophiopholis bakeri*
              - *Ophiopholis longispina*

            - Family AMPHIURIDAE
              - *Pandelia carchara*
              - *Unioplus strongyloplax*
              - *Unioplus macraspis*
              - *Axiognathus squamata*
              - *Axiognathus pugetana*
              - *Diamphiodia periercta*
              - *Diamphiodia occidentalis*
              - *Amphiodia urtica*

Ophioscolex corynetes (Clark) Th. Mortensen, 1933. Vidensk. Medd. Naturhv.

Ophiocynodus corynetes H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 274.

Geographical range — Washington, Oregon (Clark, 1911, 1915; Astrahantseff and Alton, 1965), California (Hartman and Barnard, 1958). Bathyal, 300–685 fath.

Local distribution — Outer coast of Washington (Clark, 1911). Bathyal, 685 fath.

Substrate — Mud (Clark, 1911).

SUBORDER Euryalina Lamarck, 1816
Family ASTERONYCHIDAE Muller & Troeschel, 1842

Asteronyx loveni Muller & Troeschel, 1842. Syst. Ast. p. 119.

Geographical range — Norway, Scotland, Japan, east and west coasts of the United States, Alaska, British Columbia, and the Philippine Islands (Clark, 1911, 1915; Koehler, 1922; Lyman, 1878). Bathyal, 83–918 fath.

Local distribution — Outer coast only (Clark, 1911, 1915). Bathyal, 83–918 fath.

Substrate — green mud (Clark, 1911, 1915).

Feeding behavior — Juveniles are bottom-dwelling detritus feeders; adults mount pennatulids to catch zooplankton (Hyman, 1955; D'yakonov, 1967).

Gorgonocephalus caryi (Lyman, 1860) Lyman, 1881. *Challenger* Oph. p. 264.

Astrophyton caryi Lyman, 1860. Proc. Boston Soc. Nat. Hist. 7: 424.

Astrophyton stimpsoni Verrill, 1869. Proc. Boston Soc. Nat. Hist. 12: 388.

Gorgonocephalus japonicus Doderlein, 1907. Zool. Anz. 25: 321.

Gorgonocephalus eucnemis (Muller & Troeschel) Doderlein, 1900. Echinodermata Olga Expt. p. 226, pl. 10, fig. 1–4 (Clark, 1911, believes this is a synonym of *G. caryi*).

Geographical range — North Pacific (Astrahantseff and Alton, 1965; Clark, 1911; Hyman, 1955). Littoral to bathyal, 5–900 fath.

Local distribution — Outer coast, Juan de Fuca Strait, San Juan Islands (Clark, 1911; Kyte, unpublished data). Littoral to bathyal, 5–900 fath.

Substrate — Rock, sand, green mud, and pebbles (Clark, 1911; Kyte, unpublished data).

Feeding behavior — Suspension feeding by hook-covered arm tendrils (Atz, 1960; personal observation).

ORDER Ophiurida Muller & Troeschel, 1840
SUBORDER Chilophiurina Matsumoto, 1915
Family OPHIURIDAE Lyman, 1865

Amphiophiura superba (Lutken & Mortensen) H. Matsumoto, 1915. Proc. Acad. Nat. Sci. Phila. 67: 77.

Ophioglypha superba Lutken & Mortensen, 1899. Mem. Museum Comp. Zool. 23.

Ophiura hadra H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 80.

Geographical range — British Columbia, Washington, Oregon, California, Mexico (Lutken and Mortensen, 1899; Clark, 1911, 1915; Astrahantseff and Alton, 1965). Bathyal, 772–995 fath.

Local distribution — British Columbia and Washington (Clark, 1911). Bathyal, 859–877 fath.

Sustrate — Green mud (Clark, 1911).

Ophiocten pacificum Lutken & Mortensen, 1899. Mem. Museum Comp. Zool. 23: 131.

Geographical range — British Columbia, Washington, Oregon, California, Gulf of Panama, Galapagos Islands (Lutken and Mortensen, 1899; Clark, 1911, 1915; Astrahantseff and Alton, 1965). Bathyal to abyssal, 501–1573 fath.

Local distribution — British Columbia and Washington (Clark, 1911). Bathyal, 786–876 fath.

Substrate — Green mud.

Ophiura cryptolepis H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 69.

Geographical range — Alaska, Bering Sea, Washington, Japan (Clark, 1911; D'yakonov, 1967). Lower bathyal to abyssal, 230–636 fath.

Local distribution — Reported only from the outer coasts of Washington from the lower bathyal and abyssal regions, 516–636 fath (Clark, 1911).

Substrate — Green mud.

Ophiura leptoctenia H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 51.

Geographical range — Bering Sea, Alaska, British Columbia, Oregon, California, Sea of Japan (Clark, 1911, 1915; Astrahantseff and Alton, 1965; D'yakonov, 1967). Littoral to abyssal, 5-1771 fath.

Local distribution — Strait of Georgia (Nielson, 1932; Clark, 1911; Ellis, 1968c), Satellite Channel (Ellis, 1967b; personal communication), Washington outer coast, and British Columbia outer coast (Clark, 1911, 1915). Sublittoral to lower bathyal, 30–876 fath.

Substrate — Strait of Georgia, fine sand (Clark, 1911); Satellite Channel, silty sand (Ellis, 1968a); Washington and British Columbia outer coast, green mud (Clark, 1911).

Feeding behavior — Omnivorous, feeding on detritus and animal food (Sokolova, 1957).

Ophiura lütkeni (Lyman) Meissner, 1901. Bronn's Theirreichs. 2: 925.

Ophioglypha lütkeni Lyman, 1860. Proc. Boston Soc. Nat. Hist. 7: 197.

Geographical range — Bering Sea and from Alaska down the American coast to central California (Clark, 1911, 1915; D'yakonov, 1967). Littoral to upper bathyal, 6–238 fath.

Local distribution — Strait of Georgia (Clark, 1911; Nielson, 1932; Ellis, 1968c), Satellite Channel and Fulford Harbor (Ellis, 1967b), Juan de Fuca Strait and the San Juan Islands (Lie, personal communication; Clark, 1911; Friday Harbor Laboratories collection records), throughout Puget Sound from Admiralty Inlet to Eld Inlet (Lie, 1968; Ting, MS, 1965; Kyte, unpublished data). Continuous from the littoral to the upper bathyal, 6–238 fath.

Substrate — *Ophiura lütkeni* is found on a large variety of substrates. It is most frequently found on sands with varying percentages of mud or silt although it has been recorded from gravel and shell bottoms (Clark, 1911; Ellis, 1968a; Kyte, unpublished data).

Feeding behavior — *Ophiura lütkeni* feeds only on materials in the sediment surface. Benthic protozoans, small metazoans, and detritus form a major part of the diet (Fontaine, personal communication; Austin, MS, 1966).

Ophiura sarsii Lutken, 1854. Vid. Medd. 1854: 101.

Geographical range — North circumpolar and common in north temperate waters (Clark, 1911; D'yakonov, 1967). Littoral to abyssal, 3–1700 fath.

Local distribution — Outer coast of British Columbia and Washington, Strait of Georgia (Clark, 1911; Nielson, 1932; Ellis, 1968c), Juan de Fuca Strait and the San Juan Islands (Clark, 1911; Friday Harbor Laboratories collection records), Satellite Channel and Fulford Harbor (Ellis, 1967b). This species of *Ophiura* does not appear south of Admiralty Inlet or on the east side of Whidbey Island. Littoral to upper bathyal, 10–238 fath.

Substrate — *Ophiura sarsii* is extremely tolerant and occurs on diverse bottoms throughout its range. However, it is most commonly found on silty or muddy sands but has been taken frequently from muds, gravel, shell, and rock bottoms (Clark, 1911; Ellis, 1968a; D'yakonov, 1967).

Feeding behavior — Primarily a predator feeding by arm hook and tube foot capture (Gentleman, MS, 1964).

Ophiomusium lymani Wyville Thomsen. 1873. The depths of the sea. p. 172.

Geographical range — North Atlantic south to Georgia. North Pacific from Alaska and the Bering Sea to the Gulf of Panama and the Dutch East Indies (Clark, 1911, 1915). Upper bathyal to abyssal, 70–1588 fath.

Substrate — Green mud and gray ooze, coarse sand (Clark, 1911).

Feeding behavior — Filter feeder (Barham et al., 1967).

Ophiomusium multispinum H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 113.

Geographical range — Washington, Oregon, California (Clark, 1911; Astrahantseff and Alton, 1965). Abyssal, 877–1050 fath.

Local distribution — The only report of this species from this area is from the outer coast of Washington from 877 fath on green mud by Clark (1911).

SUBORDER Laemophiurina Matsumoto, 1915
Family OPHIACANTHIDAE Perrier, 1891

Ophiolimna bairdi (Lyman) H. Matsumoto, 1917. J. Coll. Sci. Tokyo 38: 102.

Ophiacantha bairdi Lyman, 1883. Bull. Museum Comp. Zool. 10: 256.

Geographical range — Widespread in the Pacific and Atlantic (Clark, 1911; D'yakanov, 1967). Bathyal to abyssal, 500–1400 fath.

Local distribution — Outer coast bathyal, 636 fath (Clark, 1911).

Substrate — Mud (Clark, 1911).

Ophiophthalmus normani (Lyman) H. Matsumoto, 1917. J. Coll. Sci. Tokyo 38.

Ophiacantha normani Lyman, 1879. Bull. Museum Comp. Zool. 6: 58.

Geographical range — Widespread in the North Pacific on both sides. Outer sublittoral to abyssal, 40–1050 fath (D'yakonov, 1967; Clark, 1911, 1915).

Local distribution — Outer coast sublittoral to upper abyssal, 40–877 fath (Clark, 1911).

Substrate — Rocks and mud (Clark, 1911; D'yakonov, 1967).

SUBORDER Gnathophiurina Matsumoto, 1915
Family OPHIACTIDAE Matsumoto, 1915

Ophiopholis aculeata (Linnaeus) Gray, 1848. Rad. Animal Brit. Museum. p. 25. Includes *O. aculeata* (Linn.) var. *Kennerlyi* (Lyman) H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 133.

Asterias aculeata Linnaeus, 1767. Syst. Nat. 12 ed. p. 1101.

Geographical range — Circumboreal in distribution with a very large bathymetrical range, intertidal to 600 fath. Extends southward along the American coast to Monterey Bay, California (Ricketts et al., 1968; Hyman, 1955; Clark, 1911, 1915).

Local distribution — Occurs throughout Washington and British Columbia only in areas of rocks or shell exposed to strong and constant tidal currents. Littoral and sublittoral, intertidal to 238 fath (Clark, 1911, 1915; Ricketts et al., 1968; Kyte, unpublished data).

Feeding behavior — Primarily a suspension feeder although it will use arm loop capture for larger particles (Roushdy and Hansen, 1960; Fontaine, personal communication) (personal observation).

Ophiopholis bakeri McClendon, 1909. Univ. Calif. Publ. Zool. 6: 41.

Geographical range — British Columbia, Washington, Oregon, and California (Clark, 1911; Astrahantseff and Alton, 1965). Outer sublittoral to upper bathyal, 35–204 fath.

Local distribution — British Columbia and Washington outer coasts (Clark, 1911; Lie, personal communication), Strait of Georgia (Nielsen, 1932). Outer sublittoral and bathyal, 35–204 fath.

Substrate — Silty sand to sand and rock and shell (Lie, personal communication; Clark, 1911).

Ophiopholis longispina H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 119.

Geographical range — Washington, Oregon, California (Clark, 1911; Astrahantseff and Alton, 1965). Bathyal, 277–685 fath.

Local distribution — Reported only by Clark (1911) from the Washington outer coast bathyal, 277–685 fath, on mud.

Family AMPHIURIDAE Ljungman, 1867

Pandelia carchara (Clark) H. B. Fell, 1962a. Trans. Roy. Soc. New Zealand (Zool.) 2: 9.

Amphiura carchara H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 142.

Geographical range — Alaska, Washington, Japan (Clark, 1911, 1915). Outer sublittoral to abyssal, 60–1973 fath.

Local distribution — Outer coast, 760 fath (Clark, 1911).

Substrate — Green mud (Clark, 1911).

Unioplus strongyloplax (Clark) H. B. Fell, 1962a. Trans. Roy. Soc. New Zealand (Zool.) : 16.

Amphioplus strongyloplax (Clark) E. Nielsen, 1932. Vidensk. Medd. Naturhist. Forh. Kbh. 91: 295.

Amphiodia strongyloplax H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 164.

Geographical range — West coast of the United States (Nielsen, 1932; Clark, 1911). Littoral to upper bathyal, 2–200 fath.

Local distribution — Outer coast (Clark, 1911), Strait of Georgia (Nielsen, 1932; Ellis, 1968c), Satellite Channel (Ellis, 1967b), Hoods Canal (Kyte, unpublished data). Outer sublittoral to upper bathyal, 50–200 fath.

Substrate — Mud with varying amounts of sand and gravel (Clark, 1911; Ellis, 1968a; Kyte, unpublished data).

Unioplus macraspis (Clark) H. B. Fell, 1962a. Trans. Roy. Soc. New Zealand (Zool.) 2: 16.

Amphioplus macraspis (Clark) H. Matsumoto, 1917. J. Coll. Sci. Tokyo 38: 102.

Amphiodia macraspis H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 157.

Geographical range — Widespread in the North Pacific (D'yakonov, 1967). Littoral to abyssal, 10–749 fath.

Local distribution — Outer coast, Juan de Fuca Strait (Clark, 1911; Lie, personal communication), SE Strait of Georgia (Sylvester et al., MS 1966; Clark, 1911; Kyte, unpublished data), Port Susan (Kyte, unpublished data). Littoral and sublittoral only, 10–115 fath.

Substrate — Prefers silt or clay mud but also has been taken from sandy or sandy gravel bottom (Clark, 1911; Sylvester et al., MS, 1966; Kyte, unpublished data).

Axiognathus squamata (Delle Chiaje) L. P. Thomas, 1966. Bull. Marine Sci. Gulf Carribean 16: 831.

Amphipholis squamata (Delle Chiaje) A. E. Verrill, 1899. Trans. Conn. Acad. 10: 312.

Asterias squamata Delle Chiaje, 1828. Mem. Animals Vert. Napoli. 3: 74.

Geographical range — Found in polar, temperate, and tropical waters throughout the world (D'yakonov, 1967; Hyman, 1955). Littoral and sublittoral, intertidal to 100 fath.

Local distribution — Northern Washington outer coast (Lie, personal communication), Juan de Fuca Strait (Lie, personal communication; Kyte, unpublished data). Strait of Georgia and Satellite Channel (Nielsen, 1932; Ellis, 1967b, 1968c), San Juan Islands (Friday Harbor collections, Kyte, unpublished data), and throughout Puget Sound and Hood Canal (Ting, MS, 1965; Lie, 1968; Kyte, unpublished data). Littoral and sublittoral, intertidal to 100 fath.

Substrate — Sand, sandy gravel, sandy mud, silty mud under rocks (D'yakonov, 1967; Lie, 1968; Kyte, unpublished data).

Feeding behavior — Browsers and detritus feeders in the surface of sediments and under rocks (Fontaine, personal communication) (personal observation).

Diamphiodia periercta (Clark) H. B. Fell, 1962a. Trans. Roy. Soc. New Zealand (Zool.) 2: 16.

Amphiodia periercta H. L. Clark, 1911. U.S. Natl. Museum Bull. 75: 160.

Amphiodia peloria Bush, 1921. Puget Sound Biol. Sta. Publ. 3: 71.

Geographical range — Sea of Japan, Alaska, Washington, and Oregon (Clark, 1911, 1915; D'yakonov, 1967). Intertidal to 15 fath.

Local distribution — Garrison Bay, San Juan Island; Olga, Orcas Island (Fontaine, personal communication; Bush, 1918, 1921); SE Strait of Georgia and Deception Pass (Kyte, unpublished data), collected only in the littoral.

Substrate — Silty and sandy mud (Kyte, unpublished data).

Feeding behavior — Deposit and filter feeder (personal observation).

Diamphiodia occidentalis (Lyman) H. B. Fell, 1962a. Trans. Roy. Soc. New Zealand (Zool.) 2: 16.

Amphiodia occidentalis (Lyman) A. E. Verrill, 1899. Trans. Conn. Acad. 10: 313.

Amphiura occidentalis Lyman, 1860. Proc. Boston Soc. Nat. Hist. 7: 194.

Geographical range — Alaska to Monterey, California (Clark, 1911, 1915). Intertidal to 12.5 fath.

Local distribution — Strait of Georgia (Nielsen, 1932; Kyte, unpublished data), Juan de Fuca Strait (Kyte, unpublished data), Puget Sound (Clark, 1911), and the San Juan Islands (Johnson, MS, 1964). Intertidal to upper sublittoral.

Substrate — Under rocks in sandy mud (Kyte, unpublished data; Ricketts et al., 1968).

Feeding behavior — Mucous net suspension feeding, surface tube foot capture, and subsurface browsing (Johnson, MS, 1964; Fontaine, personal communication).

Amphiodia urtica (Lyman) A. E. Verrill, 1899. Trans. Conn. Acad. 10: 313.

Amphiura urtica Lyman, 1860. Proc. Boston Soc. Nat. Hist. 7: 195.

Geographical range — Alaska to California (Clark, 1911, 1915; Nielsen, 1932; Lie, 1968; personal communication; Barnard and Ziesenhenne, 1961). Littoral and sublittoral, intertidal to 100 fath.

Local distribution — Strait of Georgia (Nielsen, 1932; Ellis, 1968c), Satellite Channel (Ellis, 1967b), Washington outer coast and Juan de Fuca Strait (Lie, personal communication; Kyte, unpublished data). San Juan Islands (Johnson, MS, 1964), Anacortes (Sylvester and Colgston, MS, 1958; Kyte, unpublished data). Admiralty Inlet (Kyte, unpublished data), Port Madison, Central Puget Sound, Bainbridge Islands, and Eld Inlet (Lie, 1968; Ting, MS, 1965; Kyte, unpublished data). Intertidal to about 100 fath.

Substrate — Most commonly occurs on muds ranging from muddy sand to silt and clay muds (Ellis, 1968a; Lie, personal communication; Kyte, unpublished data). Young specimens have been found by the author under loose gravel on coarse sand.

Feeding behavior — Mucous net suspension feeder, surface tube foot capture, and subsurface browsing (Johnson, MS, 1964; Fontaine, personal communication) (personal observation).

DISCUSSION

The Ophiuroidea of Washington and southern British Columbia occur on all types of substrate at all depths from the intertidal down to abyssal zones. However, the distribution of specific species is determined by several biological and physical factors.

As can be seen from the above synopsis and Table 2 the ophiuroids of these regions can be classified geographically and bathymetrically. Three bathymetrical groups can be seen. One bathymetrical group is comprised of the species occurring in the bathyal and abyssal zones. The second contains those species occurring in the littoral and sublittoral zones. Those species which are eurybathic form a third group. These species are known to have an extremely wide bathymetrical range extending down into the lower bathyal

TABLE 2. The bathymetrical distribution of Ophiuroidea from Washington and southern British Columbia.

Bathymetrical region 1: Bathyal and abyssal zones (*fath*)	
Ophioscolex corynetes	345–685
Asteronyx loveni	83–900
Ophiolimna bairdi	636
Ophiophthalmus normani	40–877
Pandelia carchara	760
Ophiopholis longispina	277–685
Amphiophiura superba	859–877
Ophiocten pacificum	786–876
Ophiura cryptolepis	516–636
Ophiomusium lymani	859–1588
Ophiomusium multispinum	877
Bathymetrical region 2: Littoral to bathyal zones (*fath*)	
Unioplus strongylopax	50–200
Unioplus macraspis	10–115
Axiognathus pugetana	Littoral–100
Axiognathus squamata	" –100
Diamphiodia periercta	Littoral
Diamphiodia occidentalis	" –12.5
Amphiodia urtica	" –100
Bathymetrical region 3: Eurybathic species (*fath*)	
Gorgonocephalus caryi	5–900
Ophiopholis bakeri	35–204
Ophiura leptoctenia	30–876
Ophuira lütkeni	6–238
Ophiura sarsii	10–238

zone. However, these species are known to occur most commonly in the upper zones.

The marine waters of Washington and British Columbia can be divided into two major geographical regions according to their ophiuroid fauna. The limits of the total area included in this paper are the Columbia River on the south, 50°N on the north, the eastern-most extension of the marine environment as the eastern limit, and the offshore limit was set at 128°W due to the lack of data from further offshore. Although the two component regions are large and heterogenoeus they have a well-defined common boundary, Admiralty Inlet. The first of these regions extends north and west of Port Townsend–Admiralty Inlet, about 48°9′N, including the San Juan and the Gulf islands, the southern Strait of Georgia, Juan de Fuca Strait, and the offshore areas together with the outer coast.

The second region includes all of Puget Sound south of Admiralty Inlet, about 48°9′N, except Hood Canal and Port Susan. These latter two water bodies have not been sampled well enough to allow determination of their faunistic characteristics as data was available from only one sample in each of these two areas. This southern region consisting of Puget Sound proper is clearly defined by the presence of only five species. These are *Ophiura lütkeni*, *Amphiodia urtica*, *Axiognathus squamata* or *A. pugetana*, or both, and *Ophiopholis aculeata*. *Ophiopholis aculeata* only occurs in those areas characterized by strong constant currents such as the Tacoma Narrows. All 24 species occur in the northern or outer coast areas, or both. An illustration of the existence of these faunal regions is the distribution of *Ophiura sarsii*. Other indicators of faunistic regions and division of the regions will probably appear after further investigation of the marine environments of Washington and British Columbia.

The general morphology, reproductive behavior, and the general physiology of the Ophiuridea have been well treated by several authors, including two recent notable reviews, Hyman (1955) and Boolootian (1966). However, the distribution of the ophiuroids both bathymetrically and geographically is less readily subject to generalization, doubtless due to control by several interracting biological and physical factors. One of the most important biological factors is the feeding mechanisms of the individual species. The feeding mechanism of a particular species will interact with the physical environment determining the distribution of that species. For example, the feeding mechanisms of *O. aculeata* and *Gorgonocephalus caryi* restrict these species to areas of fast currents and solid substrates. Both geographical and bathymetrical distributions are often controlled by feeding behavior. Although the bathymetric distribution of neither of the above species is controlled by their feeding behavior, the bathymetric distribution of deposit feeders such as *O. lutkeni* is partially controlled by its feeding behavior. The feeding mechanisms, with the metabolic requirements of such species, will partially control the bathymetric distribution through dependence on the distribution of sediments and food concentrations (Buchanan, 1964; Sokolova, 1959).

Morphological adaptations to particular environmental conditions will often determine the particular mode of feeding by an individual species of ophiuroid. A good example of such a morphological adaptation is the arrangement of the salivary glands on the podia. In deposit feeders or predators such as *Ophiura* spp. the gland papillae will be poorly developed or lacking on the lateral surfaces of the podia and concentrated at the tips. The lateral surfaces of podia of suspension feeders will have well-developed gland papillae for secreting mucous threads and sheets to catch suspended particulate matter (Buchanan, 1964; Fontaine, 1965). *Diamphiodia occidentalis* is an example of a suspension feeder with the gland papillae on the podial lateral surfaces (Johnson, MS, 1964) (personal observation). Other such adaptations, both morphological and behavioral, will also help determine a species bathymetrical and geographical distribution. Study of particular adaptations such as reproductive behavior, feeding mechanisms, and other specific traits will continue to give a better understanding of the distribution of species.

KEY TO THE SPECIES OF OPHIUROIDEA OCCURRING IN WASHINGTON AND SOUTHERN BRITISH COLUMBIA

1(6) Disc and arms covered by thick skin, granules may or may not be present; a layer of plates or scales may or may not be present; arm joint vertebrae articulate by broad, hourglass-shaped surfaces enabling the arms to roll into vertical coils........ Order Phrynophiurida.

2(5) Arms slender and unbranched; thick, soft skin without granules covering the disc and arms.

3(4) Thick, soft skin covering scales and plates which become visible after drying; arm spines erect; ventral arm plates present; oral angle bearing spiniform oral papillae which resemble the teeth and tooth papillae. Bathyal regions only........ Family Ophiomyxidae; *Ophioscolex corynetes.*

4(3) Disc and arms covered by thick skin; no plates or scales, only radial shields extending to center of the disc show through; arms long and whiplike; eight or nine hook-shaped arm spines. Bathyal to abyssal........ Family Asteronychidae; *Asteronyx loveni.*

5(2) Arms richly branching from the base; disc and arms covered by skin imbedded with granules; riblike radial shields; arm spines beginning from base of arm. Littoral to bathyal........ Family Gorgonocephalidae; *Gorgonocephalus caryi.*

6(1) Disc and arms not covered by skin; plates or scales always present on arms and disc; vertebrae articulate by ball and socket joints or with interlocking processes; arm spines placed laterally on arms which are simple and move mainly in horizontal positions........ Order Ophiurida.

7(22) A pair of infradental papillae at the apex of the jaws; arms long and slender........ Family Amphiuridae.

8(9) Two oral papillae not forming a continuous row along the jaw, a gap occurs between the infradental papillae and the outer papillae, an additional papillae occurs dorsal to the infradental papillae; radial shields divergent; one tentacle scale; three terete lateral arm spines per arm joint........ *Pandelia carchara* (=*Amphiura carchara*).

9(8) Continuous row of three or more oral papillae along each jaw; radial shields various; tentacle scales various.

10(13) Four or five oral papillae.

11(12) Upper arm plates more or less rounded, little wider than long, scarcely in contact even at base of arm; oral shields large and pointed within, rounded laterally, truncate distally, about as long as wide; interbranchial spaces below practically naked with no scales; one tentacle scale.... *Unioplus strongyloplax* (=*Amphioplus strongyloplax*).

12(11) Upper arm plates more or less triangular with truncated angles, in contact with each other, at least at base of arm, rather wider than long; oral shields pentagonal or hexagonal, much longer than wide; interbrachial spaces below finely scaled; disc often swollen with orange-colored gonads and is easily lost; one tentacle scale; 4–6 arm spines; radial shields long and curved... *Unioplus macraspis* (=*Amphioplus macraspis*).

13(10) Three oral papillae.

14(17) Outermost oral papillae at least twice as long as the second.

15(16) Oral shields more elongate transversely than longitudinally; arm–disc ratio 4–5:1; arm spines equal in length to one arm joint........ *Axiognathus squamata* (=*Amphipholis squamata*).

16(15) Oral shields more elongate longitudinally than transversely; arm–disc ratio 7–8:1; arm spines one and one-half times the length of an arm joint........ *Axiognathus pugetana* (=*Amphipholis pugetana*).

17(14) Three subequal oral papillae.

18(21) Three subequal oral papillae on each side of each jaw; two tentacle scales; disc bordered with definite, flat marginal scales, sometimes bearing minute spines; no conspicuous primary scales.

19(20) Specimens collected locally are very large with arms up to 20 cm long or more; disc is large and often swollen with orange gonads and is easily lost; radial shields small and closely joined for most of their length; three terete arm spines.. *Diamphiodia periercta* (= *Amphiodia periercta*).

20(19) Smaller than *D. periercta* with flat rounded arm spines; radial shields slightly divergent with a single row of scales between them.. *Diamphiodia occidentalis* (= *Amphiodia occidentalis*).

21(18) Disc bordered with marginal scales bearing minute spines; small, conspicuous primary scales; three terete arm spines; radial shields elongated oval, tapering within and closely joined for most of their length.......................... *Amphiodia urtica*.

22(7) No pair of infradental papillae at the jaw apex, an unpaired infradental papillae sometimes at the jaw apex; arms shorter and more stout.

23(32) Arms inserted ventrally and not firmly fused to the disc.

24(27) A continuous row of oral papillae along the edge of the jaw; arm spines long and slender; disc covered with tubercles, short spines or scales, or both... Family Ophiacanthidae

25(26) Disc densely covered with granules, concealing the scales; each side of the jaw with four to six oral papillae, most distal is large and opercular; spinelets interspersed with granules on the disc; each arm joint with four to seven long, glassy spines.. *Ophiolimna bairdi*.

26(25) Disc sparsely covered with granules so that the thin scales are easily visible; each arm joint with four long spines; granules on the distal edges of the dorsal arm plates; inner oral papillae very long, distal oral papillae tooth-shaped .... *Ophiophthalmus normani*.

27(24) Oral papillae separated from the infradental papillae and not forming a continuous row; disc bearing variable spines; dorsal arm plates surrounded by supplementary pieces.. Family Ophiactidae.

28(31) Supplementary pieces very small and granulelike or pointed; disc spines complex with several points or few and long.

29(30) Radial shields large and bare; disc spines few and long; upper spines equal to about three arm joints in length................................ *Ophiopholis longispina*.

30(29) Radial shields and disc completely covered by slender, more or less thorny spines; upper spines equal to about two arm joints or less in length....... *Ophiopholis bakeri*.

31(28) Supplementary pieces large and angular; disc spines simple and coarse; arm spines several, short and thick; color is highly variable................ *Ophiopholis aculeata*.

32(23) Arms inserted laterally into the disc and firmly fused with it; usually more than 10 oral papillae per jaw.. Family Ophiuridae.

33(38) Disc not notched at the base of the arms.

34(35) Arm comb present; tentacle pores continuous entire length of arm; basal dorsal arm plates carrying a transverse row of spinules; three sharp slender arm spines.. *Ophiocten pacificum*.

35(34) No arm comb; only two to four pair of tentacle pores; arm spines short and numerous; no spinules on basal dorsal arm plates.

36(37) Two pair of tentacle pores; six to eight rudimentary arm spines; disc covered with small but distinct irregular scales; disc rounded and slightly swollen.. *Ophiomusium lymani*.

37(36) Four pairs of tentacle pores; 12–16 short, slender, pointed arm spines; disc covered with numerous, irregular, rounded, small plates more or less imbedded in the skin; disc flat, thin, and pentagonal with large radial shields... *Ophiomusium multispinum*.

38(33) Disc notched at the base of the arms.

39(40) Disc high; arms not broader than high; oral shields large and conspicuous; radial shields in contact except at the inner end.................... *Amphiophiura superba*.

40(39) Disc low and flat; arms usually broader than high; oral shields relatively smaller; radial shields more or less separated.

41(42) Thick granular skin completely covering the disc; disc notches not distinct; arm combs well-developed; seven to nine short arm spines.................. *Ophiura cryptolepis*.

42(41) Disc not covered with granular skin; disc notches distinct; arm combs various; fewer arm spines.

43(44–45) Papillae of arm comb truncated, short, and set very close together, forming a continuous row; radial shields touching only distally.................... *Ophiura lütkeni.*

44(45) Papillae of arm comb separate, short, coarse, and forming a definite comb; radial shields elongate, separated by a single row of scales throughout their entire length .... *Ophiura sarsii.*

45(43–44) Papillae of arm comb separate, fine, and acicular forming a definite comb; radial shields longer than broad, touching distally and divergent.............. *Ophiura leptoctenia.*

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Experimental Feeding of Some Medusae and Chaetognatha[1]

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The feeding behaviour of eight species of medusae was studied; total food consumed, rates of digestion, and growth rate were noted, especially in *Cyanea* and *Aurelia*.

Aurelia up to 5 cm diam can accept a wide range of size of food organisms obtained by filtering sea water through the curtain of tentacles during pulsation, whereas most other species find their food by random contact with the tentacles. In *Bougainvillia* it is the dichotomously branched oral tentacles that capture the food. The apparent selectivity of *Sarsia* in feeding on copepods is shown to be a physical effect produced by its method of swimming. *Leuckartiara* can catch and eat active organisms larger than itself. None of the medusae examined showed any reaction to artificial stimulation by a vibrating probe.

The method of transfer of food from the tentacles to the mouth is closely associated with the structure of the medusa. Long trailing tentacles contract and lift food towards the mouth and if the manubrium is long it can be manipulated to pick off the food from the tentacles. If the tentacles are short the umbrella edge folds inwards towards the mouth. If there is no manubrium, as in *Staurophora*, the cruciform stomach can accept food at any point.

Larval fish are important in the diet of most medusae and rough estimates are given of a probable consumption of 50–250 larval fish per hydromedusa, about 450–500 by each *Aurelia*, and in the order of 15,000 by *Cyanea*. A conversion factor is given for food eaten by medusae of 37%, though this is based on only few data.

Following the work of Horridge and Boulton (Proc. Roy. Soc. (Ser. B) 168: 413–419, 1967), who found that *Spadella* could be artificially stimulated to bite vibrating probes, similar experiments were made with *Sagitta elegans*. Although the chaetognaths were successfully kept alive the probes produced no feeding reaction. The methods are outlined for the benefit of others wishing to continue such investigations.

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MEDUSAE

It has long been known that medusae are active carnivores and that their presence in numbers in the plankton could have a serious effect on the recruitment of larval fish, but there are not many data available on the actual amount of food consumed. Papers on the feeding of hydromedusae are quoted by

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Russell (1953) in his descriptions of the particular species concerned, and these papers need not be quoted in full here. Especially important are those of Lebour (1922, 1923), Southward (1955) on feeding in *Aurelia*, Smith (1936) in *Cassiopia*, Thiel (1964) in *Rhizostoma*, and Krüger (1968) on the oxygen consumption in *Cynea* and *Chrysaora*.

Copepods and other small Crustacea seem to be the dominant food of most medusae but there is some evidence of selectivity. *Sarsia tubulosa* seems to feed almost exclusively on small Crustacea; *Eleutheria dichotoma* would take the copepod *Tigriopus* but not oyster larvae. Small fish are also important food: *Phialidium hemisphaericum* prefers fish to copepods, *Liriope tetraphylla* can capture fish three times its own size, and *Rathkea octopunctata* took sprat larvae even before it was liberated from the hydroid. *Laodicea undulata* at 10 mm ate young blennies one after another until the stomach was full. Although there is no evidence of cannibalism, several species will eat other medusae, ctenophores, or siphonophores, e.g. *Staurophora mertensi*, *Leuckartiara octona*, *Eutima gracilis*, *Amphinema dinema*, *Gonionemus murbacki*, and *Chrysaora isosceles*.

Medusae usually capture their prey by the adhesion of one or more tentacles after paralysis by the nematocysts and it is then transferred to the stomach. This transfer may be affected by the mouth reaching out to the food, or by the edge of the umbrella and the tentacles folding inwards towards the mouth or by various combinations of these according to the structure of the medusa. Ciliary currents are also used to transfer food and detailed studies of them are given and quoted by Southward (1955), especially for *Aurelia*.

Hardy (1956) mentioned that some medusae sense with their statocysts the presence of their prey through the vibrations produced in the water, and Horridge (1966) obtained positive feeding reactions by *Eutonina indicans* to vibrating probes. Although tests were made at the Logy Bay laboratory using the oscillator — as for *Sagitta elegans* (p. 1757) — none of eight species of medusae tested showed any reaction whatsoever at any of the frequencies or amplitudes used.

The following species of medusae were taken and their feeding behaviour observed: *Cyanea capillata* (L.), *Aurelia aurita* (L.), *Laodicea undulata* (Forbes and Goodsir), *Staurophora mertensi* Brandt, *Leuckartiara nobilis* Hartlaub, *Sarsia princeps* (Haeckel), *Melicertum octocostatum* (M. Sars), *Bougainvillia superciliaris* (L. Agassiz). These medusae showed differences in their methods of finding food and transferring it to the stomach, in the types and size ranges of food organisms acceptable, and, therefore, in their competition for food. Although the observations reported here make no pretence to fill the gaps in the known data about feeding in medusae and of their total food consumption, they do give an indication of the order of magnitude. That a small medusa of 5 cm in diameter can capture 80 larval fish in 6½ hr emphasizes the importance of obtaining more data on these lines, data which are of special importance in considering questions of recruitment of commercial fish stocks.

The medusae were kept in tanks of approximately 6 litres capacity using Greve's "planktonkreisel" (1968), but were placed in plain tanks for the feeding experiments so that there was no water circulation nor interference with visibility due to the sand in the "kreisels." These tanks had no continuous replenishment of sea water, no air bubbles, and were kept cool by standing them in a water table of freshly pumped sea water. Temperatures at sea at this period were about 9-10 C and in the laboratory about 12 C.

CYANEA CAPILLATA

This medusa has long trailing tentacles, and food is obtained only by random contact with these. A planktonic organism touching a tentacle becomes attached to it and is paralysed by the nematocysts. The tentacle then contracts, drawing the prey to the palmate lobes of the stomach which then remove the organism from the tentacle and transfer it to the stomach. Plankton touching the stomach lobes is also paralysed and transferred to the stomach.

Delap (1905) reared *C. lamarcki* in an aquarium and found that the ephyrae when first liberated fed on copepods, small medusae, and fish eggs, but later fed entirely on small jellyfishes and ctenophores.

Cyanea capillata, in an aquarium tank in Newfoundland, was omnivorous and accepted any animal material. Normally living on plankton, it accepted in the aquarium pieces of meat and poultry or even lumps of shrimp paste as well as marine organisms irrespective of whether they were active or dead.

A specimen of *C. capillata* caught by dipnet on July 9, 3 cm across, remained moribund for about a day and thereafter became active and voracious. Living specimens of the amphipods *Gammarellus homari* and *Calliopius laeviusculus* readily obtainable by dip net, and *Parathemisto gauchicaudi* from the plankton were not utilised by *Cyanea* as they "sensed danger" on touching the tentacles and their escape reaction was too rapid for the *Cyanea* to cope. Crushed specimens, however, were readily taken and the empty teguments later discarded. Euphausids and brachyuran zoeas of similar size were eaten, as were other medusae, or cut portions of medusae.

Apart from the experimental material given to test acceptability, this specimen was kept on fresh *Mytilus edulis* and on living *Temora* and larval fish. In 17 days it consumed about 500 *Temora* (wet weight approx 0.032 g), 6 flatfish larvae 12.5–15 mm long (wet weight 0.358 g), and pieces of *Mytilus* weighing 0.15, 0.28, 0.37, 0.54, 0.75, and 1.23 g, and grew to 5 cm in diameter. At first the *Mytilus* given was chopped to about the size of a *Calanus* but later much larger pieces were given and whole *Mytilus* extracted from shells of about 3 cm long were readily taken.

When heavily laden with food, *Cyanea* would "rest" on the bottom of the tank, but when the food had been partly digested *Cyanea* became active again.

Growth from 3 to 5 cm incurred an increase in wet weight from 1.75 to 8 g and to do this it consumed 3.7 g of food. Its own body is approximately 96.47% water (Koizumi and Hosoi, 1936) as against 84% for its food, an approximation based on the figures for dry weight for *Mytilus* as 16.66%, and 14% for *Temora* and fish larvae (Vinogradov, 1953). The growth of *Cyanea* in dry weight is about 0.22 g and its food is 0.59 g, making a conversion factor of 37%. These figures can only be regarded as very approximate, as one specimen was involved and it is not possible to get accurate wet weights of a medusa and still keep it alive and in good condition. Nevertheless they are probably of the right order, as Krüger (1968) has also shown a fast growth rate accompanied by a high oxygen consumption in the Scyphozoa. These results imply that an adult *Cyanea* of 45 cm diam weighing about 6 kg would have a dry weight of 212.5 g; it would require 570 g dry weight of food or approximately 3570 g wet weight. This would be the equivalent of about 1.66 million *Calanus* in its life. *Cyanea* frequently reaches 1 m in diameter and very large arctic specimens are reported to reach about 2 m diam.

AURELIA AURITA

Southward (1949, 1955), Orton (1922), Gemmill (1920), and others have stressed the great importance of ciliary currents in the feeding of adult *Aurelia* although the young stages feed by both a ciliary mechanism and by muscular macrophagy. Lebour (1922) records that ephyrae of *Aurelia* only a few days old would eat larval fish and continue to do so until at least 25 mm in diameter. Figure 1 is taken from a photograph and shows a herring larva in the stomach of *Aurelia* only 5 mm in diameter taken in the Scottish plankton. In her 1923 paper Lebour says that *Aurelia* more than 30 mm across were usually empty and that it is possible that the larger food, such as fish, is only taken in the younger stages. Southward (1955) states that the difference in feeding habits between the ephyra and the adult is due merely to the growth of the jellyfish: the food organisms are only relatively smaller in the adult stage.

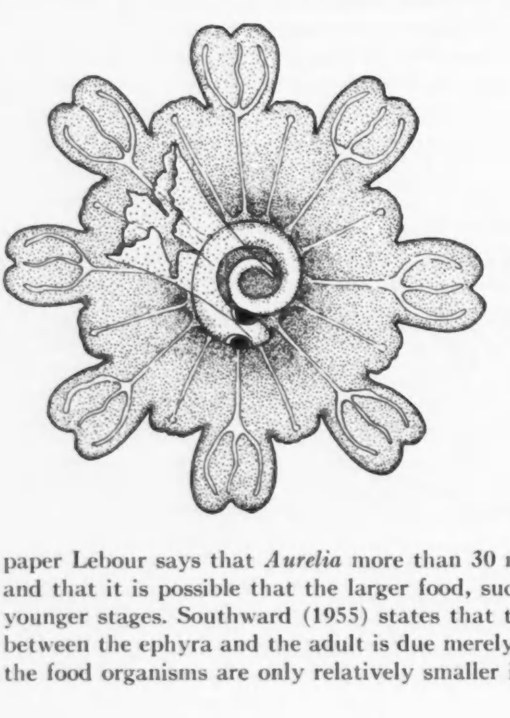

FIG. 1. *Aurelia aurita*, 5 mm in diameter, with a larval fish in its stomach.

Delap (1907) stated that the ephyrae of *Aurelia* had a preference for small medusae, such as *Obelia*, and that they were difficult to rear as it was "sometimes impossible to get the food that they like," yet Delap also says that "Above all they like variety; two or three days with only one kind of food quite upset their digestion."

This is not in accordance with the findings at Newfoundland in 1968, described below, where a specimen 20 mm in diameter, wet weight about 0.5 g, was taken on June 19, 1968. Normally feeding on planktonic organisms it would accept in the experimental conditions any animal material. There seemed to be no preferences for food as it was caught only by random contact.

Feeding at this size seemed to be less by the ciliary currents than by a filtration mechanism (Fig. 2) through the fine meshes of the tentacles of which there are about 500. The tentacles almost meet in the centre of the umbrella when it starts to expand (Fig. 2, 4) and extend well beyond the umbrella as it starts to contract (Fig. 2) so that a short column of water rather more than the maximum diameter of the umbrella is sampled. When *Aurelia* is 50 mm in diameter each pulse propels it about 4 cm and it thus filters about 22 ml per pulse. The pulsation rate is almost exactly 1 per sec so that it filters at this size about 132 ml per min or 1 litre in 7½ min.

When food is captured it is paralysed by the nematocysts on the tentacles and transferred to the edge of the umbrella which then bends inwards slightly at this point; the lips of the stomach then reach out to meet it. The food is transferred by a wiping movement as the tentacles and edge of the umbrella are withdrawn. This process takes about 45 sec but is slower if the medusa is well fed. It continues to pulsate throughout this process. The food takes about another minute to reach the top of the manubrium and so into the stomach proper. There it is pushed to the outer edge of one of the four horseshoe shaped gastric pouches which lie against the gonads, so that a stomach packed with copepods resembles a small but fully mature specimen.

Themisto and other amphipods were ingested but later discarded whole as the digestive juices did not seem able to penetrate their integuments, but broken amphipods were digested and their empty integuments discarded. Other medusae were readily taken by *Aurelia*. A *Laodicea* of 15 mm first touched the tentacle of a 20-mm *Aurelia* and was inside the stomach, whole, 7 min later. The contents of *Mytilus* 3 cm in shell length were easily taken whole and completely digested overnight. Yolk-sac larvae of *Gadus saida* and American flounder were digested in about half an hour except for the eyes which took about 2 hr to disappear.

Planktonic copepods, chiefly *Temora*, and fish larvae (cod, poor cod, and American flounder) were used as normal food, *Mytilus* being given when copepods or fish larvae were not available, and *Aurelia* continued to grow reaching 5 cm diameter in a month. During this time it consumed 2 *Laodicea* (12 and 15 mm, 0.40 g), 4 crushed *Themisto* (0.12 g), 4 *Calanus* adults (0.01 g), 240 *Temora* and *Pseudocalanus* (0.02 g), 4 zoeas (0.05 g), 3.5 g of *Mytilus*, 112 larval fish (yolk sac and immediate post yolk sac 0.25 g), 4 fish eggs,

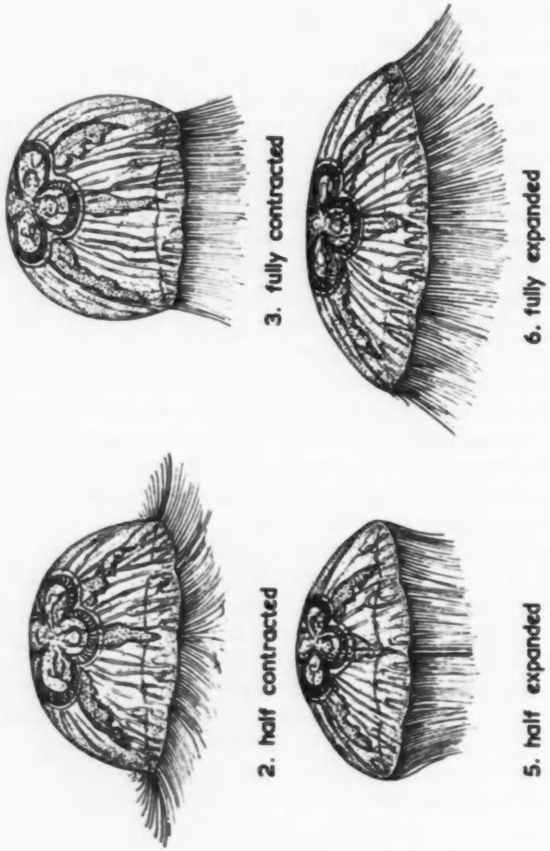
1. starts to contract
2. half contracted
3. fully contracted
4. starts to expand
5. half expanded
6. fully expanded

FIG. 2. *Aurelia aurita*, showing movement of the tentacles during pulsation.

and 3 larval fish of 12–15 mm (0.15 g). Kept in a tank of 6 litres it caught all 80 larval fish, added in batches of 20 at hourly intervals, in 6½ hr when its own diameter was 5 cm and its wet weight 6.64 g.

After a starvation period of 10 days, *Aurelia* was still alive but sluggish in movement and reduced in size to about 4 cm; it quickly recovered on being fed but it would no longer accept whole *Mytilus* from shells of more than 2 cm long. Smaller pieces, whole decapod larvae, and crushed amphipods of fully 1 cm long seemed acceptable but were not pushed into the stomach proper and were held by the four lips at the mouth where digestion occurred. Its former size of 5 cm was regained in about 1 week.

It thus seems that *Aurelia* can feed on relatively large organisms from the plankton until it is about 50 mm in diameter, and that some of these can be considerably larger than can be taken by the adult, which is microphagous (Southward, 1955).

The specimen kept in the tank at Logy Bay increased in weight from approximately 0.5 g to 6.64 g wet weight. It consumed 4.5 g wet weight of food. Figures for the percentage of water in *Aurelia* given in the literature (e.g., see Hyman (1938, 1939, 1940) and Vinogradov (1953)) range from 95.3 to 98.4, the higher figures being in water of very low salinity and 95.5% is used here to convert the increase of 6.14 g to 0.275 g dry weight. Taking the food at 84% water its dry weight would be 0.72 g, a conversion factor of 37% as shown above for *Cyanea*, although again based only on one specimen and on only approximate wet weights of the living medusa.

In proportion an adult *Aurelia* of 25 cm would weight 830 g (3.75 g dry wt) and eat 610 g of food (9.7 g dry wt) which would be equivalent to about 60,000 *Calanus*. Even if it is assumed that no fish were taken after 50 mm diam and that about one-quarter of its food until that size was in the form of yolk sac and early larval fish, and using the weights of the fish given in this experiment, an *Aurelia* could consume about 450 young fish in its early stages.

LAODICEA UNDULATA

Lebour (1922, 1923) mentions that *Laodicea* taken in the plankton contained many *Calanus* and one specimen, which survived only 1 day in the aquarium, ate several young blennies in succession.

Only small specimens of *Laodicea* were taken alive at Logy Bay. One, 7 mm diam, was taken on June 19. Examined microscopically, it was seen constantly to wipe its tentacles on the lips of the manubrium, taking off small fragments of detritus, ciliates, and minute oil globules from *Coscinodiscus* which was abundant in the plankton at that time. Most active copepods, copepodites, and nauplii were able to dodge this small medusa but there was an occasional successful capture by random contact and entanglement.

Deliberately presented with a pandalid larva, euphausid calytopis and a stage IV *Calanus* it took all together and continued to wipe its tentacles every few minutes. The euphausid, though ingested, was not paralysed and continued to struggle for 10 min, but the pandalid was more easily digested and only the eyes were seen after 12 min. *Coscinodiscus* were ingested and digested if they touched the lips of the manubrium but they did not stimulate any feeding reaction when they touched the tentacles. The pulsation was almost continuous at a rate of 140 per min.

About 10 mm in diameter on July 4, it had captured and digested in the previous 6 days 5 larval fish (immediately post yolk sac) and 21 copepods (mostly *Temora*). The edge of the umbrella (presumably due to some damage) was attacked by ciliates (*Euplotes* sp.) on July 9 and these rapidly multiplied on the dead and dying material while the rest of the umbrella continued to pulse and accept *Temora* as food. Only two radial canals were left by July 10 and on July 11 about one-third of the umbrella was left, although most of the manubrium remained intact, still pulsating and accepting food. It did not die, very shrivelled, until July 15.

Two other specimens failed to survive in the laboratory and two others put into the same tank as the *Aurelia* were eaten by it.

STAUROPHORA MERTENSI

This was the most abundant medusa in the vicinity of Logy Bay in the summer of 1968 and specimens from 15 to 164 mm diam were taken, the most common size being from 90 to 120 mm. It is somewhat hemispherical in its early stages but later becomes very flat indeed. Its movements are slow and erratic, pulsing about once in 5 sec, often much more slowly, but small specimens during active periods can pulsate about 1 per sec. *Staurophora* has no manubrium; the stomach is distributed along the four radial canals, almost as far as the edge of the umbrella and the lips extend the full length of all four arms of the stomach. The very numerous tentacles are distributed along the whole edge of the umbrella, but are not as long as those of *Aurelia* of the same diameter, and even in the arched early stages they do not meet centrally as the umbrella starts to expand. Filtration is therefore not efficient and capture is more by random contact than by filtration although the whiplike waving of the tentacles does mean that a moderately large volume of water is "searched" for food and this is more effective in the smaller than in the larger and flatter specimens.

When food is caught by the tentacles they contract until the food reaches the margin of the umbrella which folds in at this point until it pushes the food against the hanging lips of the stomach which stretch out somewhat to meet it (Fig. 3). Food is ingested and digested where it reaches the stomach and is not centralised; this was noted also by Agassiz (1849). A fish larva, if caught by only one end, does not immediately produce the infolding reaction but the medusa continues to pulsate, its tentacles waving until the larva is caught throughout its length.

A specimen of 15 mm diam was taken on June 19 and successfully caught eight fish larvae (immediately post yolk sac) in a few hours, each taking about 45 min to digest. It remained alive for only 4 days, growing to 23 mm

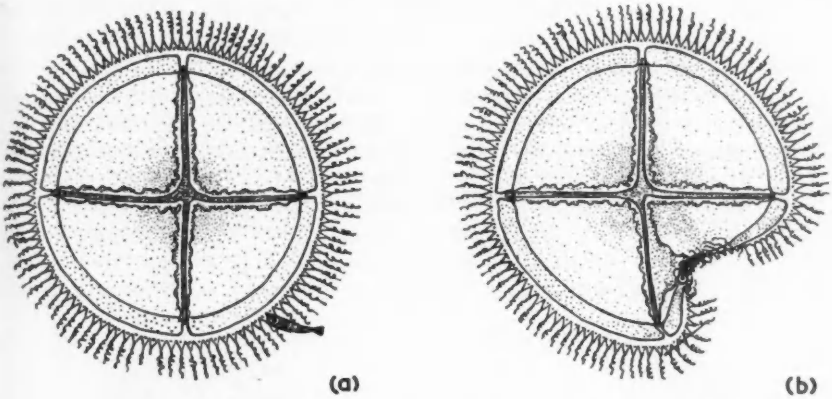

FIG. 3. *Staurophora mertensi*, showing method of transfer of food to the stomach.

in diameter, and during this time consumed 13 fish larvae, 47 copepods, 2 crushed amphipods, and 0.1 g of *Mytilus*. After a surfeit of *Mytilus* it would not accept more but accepted a crushed amphipod.

A further specimen, dipnetted July 11, grew from 25 to 40 mm by July 26, consuming 2 crushed amphipods, 21 fish larvae, 200 copepods, 2 zoeas, and 0.29 g of *Mytilus*. It would not accept *Mytilus* bits if there was other food in the stomach.

The large specimens would accept *Mytilus* taken whole from shells up to 3 cm long but no large specimens survived in the laboratory for more than 4 days.

LEUCKARTIARA NOBILIS

Lebour (1922, 1923) studied the feeding of an allied species, *L. octona* (as *Turris pileata*) and found it to be highly voracious even swallowing food larger than itself, including small fish. It is thus dangerous to keep in an aquarium.

The species taken in Newfoundland waters in 1968 was *L. nobilis* and I am indebted to Dr C. Edwards for his help in this identification. Its appetite seems essentially similar to that of *L. octona*.

Several specimens were kept alive in tanks in the laboratory. In this species the tentacles, about 40, trail behind the slowly pulsating bell. There appear to be no whiplike movements in the tentacles and capture of food is entirely by random contact. The tentacles themselves often coil and twist and thus become entwined into complicated knots; in such instances the medusa will discard the entangled balls of tentacles rather than unravel them. This can happen several times in the course of a week. Larval fish or copepods becoming entangled sometimes take a comparatively long time to reach the stomach — from several minutes to a few hours. At other times the reaction is much faster, but once the food reaches the umbrella edge after contraction of the tentacles the edge of the umbrella immediately curves strongly inwards and conveys the food to the stomach in a few seconds.

Leuckartiara will take surprisingly large food organisms such as *Sarsia* (Fig. 4), other medusae which are as big as itself, and one specimen only 17 mm bell height (i.e. excluding the dome) very rapidly caught and swallowed *Clione limacina* 25 mm long. In such instances the whole manubrium expands to cover the food organism, pushes it into the stomach which distorts to accept it while the manubrium contracts to its static position (Fig. 4*b*). The *Sarsia* could be seen attempting to pulsate and the *Clione* to wriggle inside the stomach for several minutes. Unless actual contact is made there is no reaction by the medusa to fish, Crustacea etc. actively swimming close beside it.

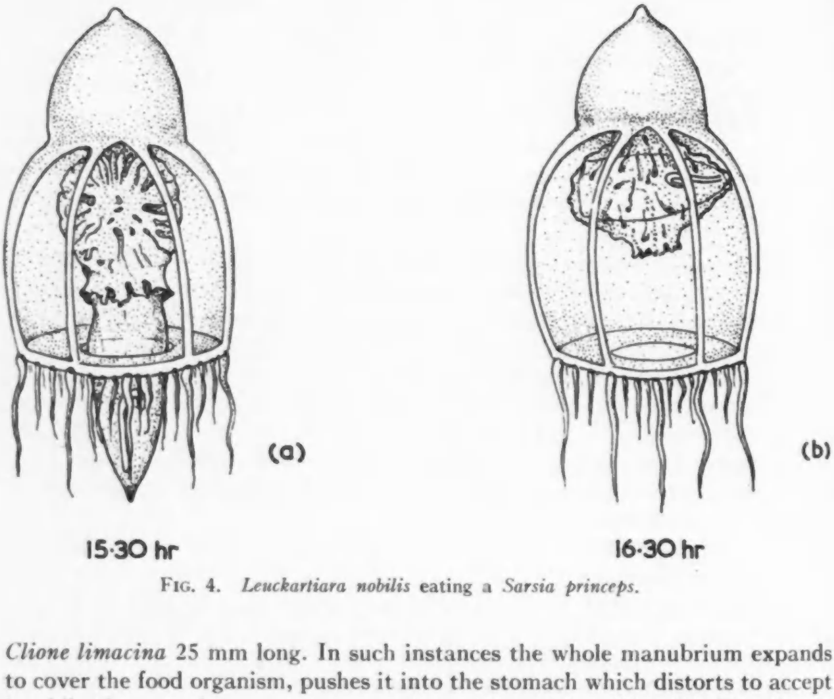

FIG. 4. *Leuckartiara nobilis* eating a *Sarsia princeps*.

This specimen (17 mm) caught on July 9, was fed until August 3, and in this period of 26 days consumed 22 fish larvae (yolk sac and post yolk sac), 222 copepods (mostly *Temora*), 4 amphipods, 0.875 g of *Mytilus* given in 10 applications, 4 *Aglantha*, 1 *Sarsia*, and 1 *Clione*.

After starving for 10 days, it had changed from a bright orange colour to a deep crimson, and although it accepted some food, it died 3 days later.

Other specimens provided similar results and they would accept pieces of umbrella cut from dead *Staurophora*.

SARSIA PRINCEPS

An allied species, *S. tubulosa*, was kept by Lebour (1922) for about 10 days. She reported that it was constantly eating copepods but never anything else. At Logy Bay the species caught was *S. princeps* (identification confirmed by Dr C. Edwards). This species (like *S. tubulosa*) has a long very pointed bell, four long tentacles and an elongated manubrium which can extend well beyond the end of the bell. It pulsates powerfully but rather erratically, shooting forward rapidly, trailing the manubrium, the four tentacles undergoing whip-like convolutions in the turbulence of the slipstreams. If a copepod or other suitable food touches a tentacle, it adheres, is paralysed, and the tentacle lifts it towards the mouth which is extended to meet it (Fig. 5*a*). The food is encircled by the very elastic mouth and lifted off the tentacle. Food touching the manubrium causes this to encircle it boa-constrictor fashion, and the mouth turns upwards to engulf it (Fig. 6*b*), a feature seen by Lebour (1923) in another species with an elongated manubrium, *Saphenia gracilis*. *Sarsia* exhibits no response reaction except when food actually touches it.

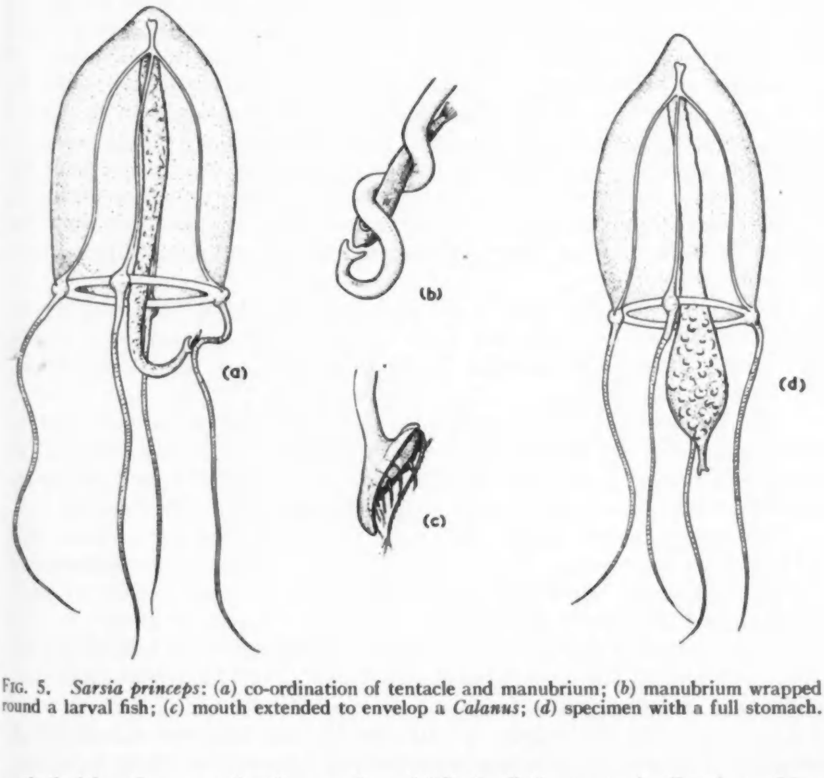

FIG. 5. *Sarsia princeps*: (*a*) co-ordination of tentacle and manubrium; (*b*) manubrium wrapped round a larval fish; (*c*) mouth extended to envelop a *Calanus*; (*d*) specimen with a full stomach.

Pulsation usually continues in vigorous bursts during the feeding process so that anything bigger than a copepod is washed off, and copepods not properly adhering are also washed off. Loss can also occur during the transfer of food from the tentacle to the mouth. During periods of relaxation in the aquarium dish *Sarsia* would cease to pulse and sit upright on its four tentacles with the manubrium pressed to one side; during these periods it would accept pieces of *Mytilus* or other food placed very close to the mouth. Touching the bell, manubrium, or tentacles would, however, stimulate the bell to pulsate and the food would be lost. A live and active *Poecilochaetus serpens* produced no effect whatever on *Sarsia* even when actually touching the tentacles.

Food is digested in the stomach quite near to the mouth and the manubrium can be very extended at this part if *Sarsia* is actively feeding on abundant sources of food (Fig. 6*d*). After digestion *Sarsia* ejects a faecal pellet containing the undigested remains. Fish larvae continue to wriggle inside the stomach for a while so that the paralytic effect is not strong, and they take about 1½ hr to digest. Experimental evidence suggests that such fish larvae could not have been caught by *Sarsia* under natural conditions as during a relaxed period they would not become entangled by the tentacles and during an active period they would be washed off. If different food organisms become attached to more than one tentacle at the same time, only one lifts, the others continuing to trail until the mouth has ingested the food from the first tentacle. The second then lifts and the mouth extends to meet it, the meeting being very accurately accomplished.

An active *Sarsia* was kept in the same dish as a slowly pulsating *Staurophora* of about the same size and 4 fish larvae and 27 copepods were added. *Sarsia* took 20 copepods whereas the *Staurophora* caught all the fish larvae and only 7 copepods.

The approach of senility in *Sarsia* is seen in the reduction of the manubrium and loss of the mouth. No feeding can occur and *Sarsia* continues to swim about actively for a while, shrinking all the time and gradually becoming moribund until death about a week later. Four specimens were lost this way.

One specimen, bell height 11.5 mm, was taken on June 18 and until the loss of the manubrium on July 16 it consumed 5 fish larvae (deliberately given), 59 copepods, and 0.135 g of *Mytilus*. Another taken on July 11 took until August 3 240 *Temora*, 14 fish larvae, and 0.43 g of *Mytilus*. It then existed for a 10-day period of starvation but although it accepted a little food thereafter it never recovered. The tentacles and manubrium were all extremely thin and extended to their maximum length.

It would seem from these experiments that the apparent selectivity of copepods by *Sarsia* is not a matter of differentiation of the food by sense, but by the inability of the *Sarsia* tentacles to retain hold of anything larger than a copepod whilst it is actively pulsating. Some of the fish larvae lost had been adequately paralysed and were dead so that wriggling by the fish larvae is not responsible for their loss.

BOUGAINVILLIA SUPERCILIARIS

This is a very round species with short tentacles arranged in four groups: there is a distinct velum which partially closes the arched umbrella. The manubrium dichotomously branches and rebranches and these dendritic oral tentacles are used to capture food which enters the bell either accidentally or is sucked in during pulsation. The tentacles appear to take no part whatsoever in finding or securing food which was therefore confined to small zooplankton organisms. Lebour (1923), however, figures a very young *B. britannica* only 1 mm across attempting to eat a larval sandeel of about 5 mm. Active copepods entering the bell can usually avoid capture and dart out again but occasionally one will dart too far into the bell and become entangled in the constantly waving branches of the stomach. Nauplii and early copepodite stages are more readily caught. When food becomes entangled in the manubrium the bell contracts at the opening so that the velum almost, but not quite, closes the exit (Fig. 6). Copepods continued to wriggle inside *Bougainvillia* for 2 or 3 min. Inert animal material such as pieces of *Mytilus* were readily accepted but not small pieces of other medusae. One specimen, caught on July 30, took 6 nauplii and 3 adults of *Temora*, 2 fish larvae which were placed inside the bell, and a piece of *Mytilus*, 0.01 g, but it failed to survive a 10-day period without food.

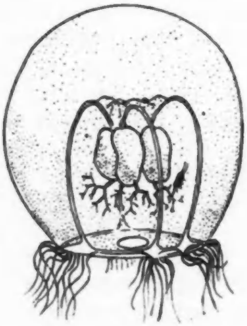

FIG. 6. *Bougainvillia superciliaris* with velum almost closed to retain a copepod.

MELICERTUM OCTOCOSTATUM

One specimen (Fig. 7) was taken in the plankton on July 30. It is a small species, bell height 10 mm and lemon yellow in colour. When swimming it usually, but not always, pulsated twice in rapid succession, at about 1-sec intervals, followed by a pause for about 3 sec, its tentacles usually being kept extended outwards at about right angles to the bell and much as figured by Mayer (1923, plate 23, fig. 5) under the name *M. campanula*. The tentacles, however, trail after it as the bell starts to expand after pulsation. When the stomach is full it will pulsate to swim with its tentacles closely curled. Feeding appears to be entirely by random contact and it successfully captures active

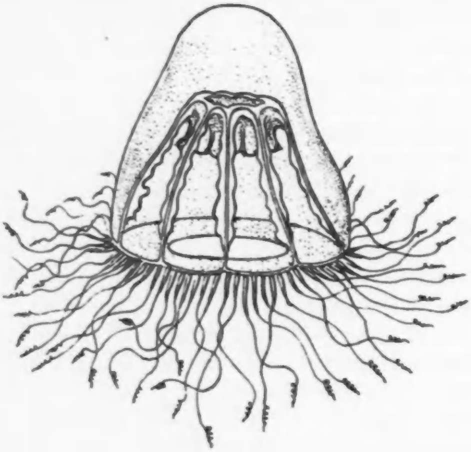

FIG. 7. *Melicertum octocostatum.*

plankton like young fish larvae and copepods but will also accept inert food such as pieces of *Mytilus* or parts of other medusae. Kept in a dish of about 1 litre it caught 4 out of 5 larval fish added in 10 min and later 6 out of 10 in 20 min. During a 4-day period it consumed 35 fish larvae, 6 *Temora*, and a peice from *Staurophora*. Thereafter it survived 10 days without food and continued to be very active after feeding on *Mytilus* and attained a bell height of 12 mm.

EFFECT OF MEDUSAE ON THE RECRUITMENT OF LARVAL FISH

As medusae have long been known to feed to some extent, and often voraciously, on larval fish it has been suspected that they could have serious effects on larval mortality and consequently on stock recruitment. Most fish lay large numbers of eggs, figures of several thousands to a few millions are common, and they do so each year for several years of maturity. To maintain a steady stock only a male and a female need survive through to the adult stage, so that mortalities of 99.99 to 99.9999% are quite usual and only slight differences to these make big differences in the survival rate and stock recruitment. The part played by medusae in this, although suspected, is not adequately known. The high mortality of fish larvae is due in part to lack of adequate food at the right time, and in part to larvae of demersal fish being carried to unsuitable areas for later settlement, but predation is also a vital factor. Medusae, although important, are not the only predators.

The figures given on p. 1745 imply that a *Cyanea* of 45 cm diam will have consumed the equivalent of about 1½ million *Calanus*. A yolk-sac stage fish is not too different in biomass from a *Calanus*, and if only $\frac{1}{100}$ of *Cyanea's* food is fish larvae this could mean a total consumption of some 15,000 fish during its life. It is probable that $\frac{1}{100}$ is a conservative estimate, but a proportion of the fish will be larger than the yolk-sac stage, so that the suggested 15,000 is at least likely to be of the right order.

Aurelia, which is more abundant than *Cyanea*, includes young fish in its food until it is about 6 weeks old, and one such specimen actually caught and ate 80 larval fish in 6½ hr. Only a proportion of its food in the sea will be fish, but allowing for this it could be reasonably estimated that the expected consumption would be 400–500 fish larvae for each individual *Aurelia* in these 6 weeks. The smaller hydromedusae, which are more abundant than either *Aurelia* or *Cyanea*, could in proportion be expected to eat 50–250 fish larvae each. The resultant average might thus be estimated between 100 and 200 fish larvae per medusa.

The numbers of fish larvae eaten by each medusa will depend on the abundance of other food. For example, where copepods abound the proportion and quantities of young fish in the total food may be expected to be less, and the predation on fish less drastic, even though the numbers of medusae or fish were the same as in areas of fewer copepods. The numbers of larval fish eaten will also be very dependent on the timing and the distribution of the hatching fish larvae, relative to those of the medusae. With the consumption of fish larvae by each medusa varying from 50 to 15,000, there could be major yearly differences in total consumption, which would not be revealed by investigations on medusae alone, yet which could be very significant for the fish stocks and thus for the fishing industry.

Thus, although there seems to be no doubt that very large numbers of potential fish recruits are lost to medusae, one cannot expect a simple mathematical relationship between the two population numbers. The more medusae produced the more likely there is to be a decrease in fish survival, and it could be a serious decrease, but such a decrease need not be associated with an increase in the population of medusae even though they might be responsible for it. It might well be governed by changes in other circumstances.

CHAETOGNATHA

Horridge and Boulton (1967) reported that the bottom-living chaetognath, *Spadella*, would give a positive feeding response and bite a glass probe vibrating between 9 and 20 cycles/sec and at an amplitude of 100–500 μ if the probe was placed within 1–3 mm, thus confirming the generally accepted theory that they were sensing the vibrations caused by the swimming movements of their prey. The eyes of chaetognaths are not constructed to give any reliable visual image (Tokioka, 1950; Eakin & Westall, 1964), yet they actively capture their prey, even in the dark (Parry, 1944; Reeve, 1964). Data on the appetites and rates of digestion of the planktonic chaetognatha are much needed as a means to assess the predation of these carnivores in the zooplankton and on larval fish in particular. Lebour (1923) says that a large proportion of the specimens taken in any haul are empty but so many are eating herring that much damage must be done. If the Horridge and Boulton technique could be applied to *Sagitta* this could give a method of artificially stimulating feeding behaviour leading to the acceptance of known amounts of food at set times. Results of such work could give valuable additional information to that already obtained from a study of the food of *Sagitta* as seen in the stomachs of captured specimens (see especially Lebour (1922, 1923), Wimpenny (1937), and Della Croce (1963) and in feeding experiments (see Parry (1944) and Reeve (1964).

Sagitta elegans Verrill was the only chaetognath taken in the waters near the southeast coast of Newfoundland during the period of this work. It occurred only rarely during June and early July but abundantly from the second half of July onwards. It is a delicate species and, after sorting in the laboratory as soon as possible the material taken in a 75-cm plankton net, and within about an hour of landing, most of the individuals were dead. Of those still active enough to attempt to avoid capture by pipette only about 5% survived overnight, but having done so they survived well thereafter in glass dishes kept cool in the laboratory by floating in a water-table with a continuous flow of freshly pumped sea water.

After 5 days starvation many free copepods and copepodites of various stages were added to the dish containing the *Sagitta* but no copepods were seen in the stomachs, even in the early morning. Parry (1944) estimated about 5 hr for digestion in *Sagitta setosa* so that remains of any food taken during the night hours should still have been visible even if feeding stopped at dawn. That natural feeding was not achieved may have a bearing on the failure to produce positive feeding reactions in Newfoundland using vibrating probes. Nevertheless, as a contribution to further experimental work it is considered advisable to describe the methods used and details of the resulting escape reactions.

TECHNIQUES

By courtesy of the Physics Department of the University an oscillator was made available; Bryston Manufacturing Ltd, Scarborough, Ontario, Type 4024, 115 v, 60 cycles/sec, 60 w output at 600 ohms, sine wave form. This was connected through a 600-ohm resistor to a 35-ohm loudspeaker, 2½ inches (63 mm) diam, flux 8000 *g*. Probes were made of glass and attached by fine plastic tubing to be in contact with a plastic rod 1 mm diam fixed to the cone of the speaker by "Araldite." Frequencies from 8 cycles to 1.5 Mcycles/sec were available with voltage control to adjust the amplitude. The probes used had the shapes illustrated in Fig. 8. Because of the resonance characteristics of both the speaker and the probes, the amplitude at the tip of the probe in sea water was observed under a stereo microscope and adjusted for each change of frequency. At lower frequencies the probes oscillated to and fro but at frequencies above 50–70 cycles/sec the movement became lateral. The amplitude of the probes with globules at the tip could readily be measured by the movement of the globules, but the point of resonance of the pointed probe varied according to the frequency and the point of maximum movement was measured. Observations on the reaction of *Sagitta* were carried out by naked eye in small dishes of about 200-ml capacity and in watch glasses under the stereo binocular microscope.

RESULTS

There was an escape reaction, which varied from a slight wriggle to a violent movement as soon as the probe touched the surface film of the dish, if the *Sagitta* was less than about 1 cm away, and this occurred with inert as well as activated probes. Thereafter, carefully held inert probes produced no reaction unless they actually touched the *Sagitta* when the escape reaction was sudden and vigorous. After only two or three repetitions there was no further reaction on touching the surface film and after 6–10 contacts no reaction on touching the *Sagitta*. Initial reactions could then be obtained again after a wait of 2–3 min. *Sagitta* could be chased by a moving inert probe, or a pipette, when there would be effective avoidance as soon as a current was produced by the suction of the pipette.

Activated probes when steadily held produced an escape reaction not apparent with inert probes. Tests were made using 10 individual specimens of *S. elegans* 7–16 mm long and the reactions of each were essentially similar. Owing to the sudden motion of *Sagitta* it was not possible to see by naked eye whether the escape reaction first included a bite attempt, but details of the movements were clearly seen under the low power microscope and in none of these was any positive bite reaction seen, only escape reactions.

Tests using an adjusted amplitude of 200 μ at the tip of probe 1 (Fig. 8) were made at 10, 12, 13, 15, 20, 22, 25, 30, 35, 40, 50, 60, 70, 80, 90, and 100 cycle/sec. An individual *Sagitta* was not kept under the microscope for more than 5 or 6 min at a time; thereafter, it was allowed a rest of at least half an hour. At frequencies up to 30 cycles/sec the escape reaction first occurred when a probe was about 0.5 cm from the *Sagitta*, 35–40 cycles/sec about 1 cm, 50–60 cycles/sec 1.5 cm, dropping back to about 1 cm at 80 cycles/sec. Tests made with adjusted amplitudes of 100 and 50 μ were made over the same range of frequencies and reactions were similar to those to an inert probe. Increasing the amplitude to 500 μ caused escape reactions too rapid to be

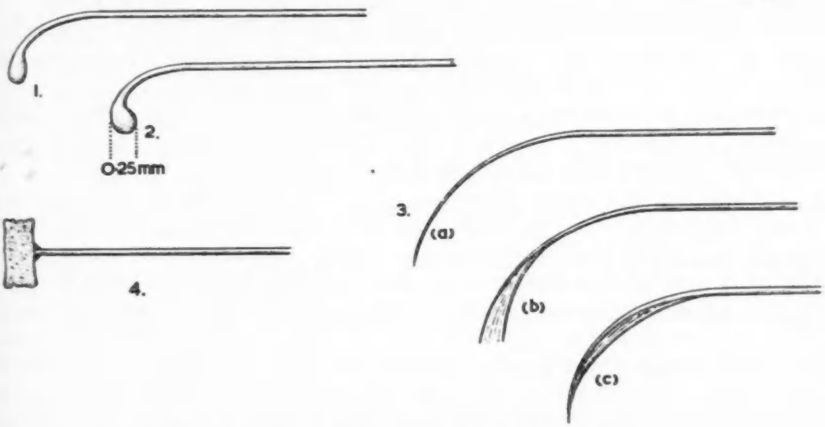

FIG. 8. Types of probes used with the oscillator.

seen in detail, *Sagitta* sometimes breaking the surface film. Frequencies greater than 100 cycles/sec produced water turbulence and the currents so produced masked any gentle motion of the *Sagitta*. Experiments with probe 2 (Fig. 8) produced similar results to those with probe 1.

The most abundant copepod in the plankton at this time was *Temora longicornis*, which was available as adults and as copepodite stages. These were normally swimming about in a gliding fashion using only the rhythmic movements of the pleopods. This rate was measured by adjusting the frequency of an oscillator probe to match, an eye estimate easily giving sufficiently adequate precision, and this was found to be 35 ± 3 cycles/sec. *Temora* showed no reaction whatever to the probe unless actual contact was made. It was noticed, however, that the swimming of *Temora*, *Pseudocalanus*, *Calanus*, euphausid calytopis, and *Evadne* on no occasion stimulated a positive feeding reaction by the *Sagitta* during the experiments, but a sudden escape reaction was produced when any of these Crustacea touched it.

Using the three types of probe (Fig. 8) at a basic frequency of 35 cycles/sec, but varying this experimentally in both directions, the *Sagitta* readily became accustomed to the vibrations, the reactions becoming less until after about 3 min of continuous presentation it was possible to stroke the head of the *Sagitta* gently without producing any reaction. Touching the tail would then still produce a reaction but if persisted in, the tail then became accustomed and the head would react. No positive feeding reactions were produced whether an approach was made suddenly, slowly, or persistently.

Further attempts were made to produce a positive feeding reaction after starving for periods of 5, 8, 10, and 14 days but at no time could this be achieved even if a copepod was impaled on the vibrating probe.

These negative results with *S. elegans* were disappointing and surprising considering the success with *Spadella* by this method, and in the feeding experiments by Parry (1944) and by Reeve (1964) who kept *S. hispida* alive in tanks for over 14 days feeding them on *Artemia* larvae. *Sagitta hispida*, Reeve states, ingests 64% of its own dry weight in food each day. Although many thousands of *S. setosa* and *S. elegans* from Scottish waters have been examined, very few contained food — much less than 1%. Although Lebour (1923) also found many *Sagitta* empty, other workers give remarkably large figures, e.g. Wimpenny (1937) who found about 20–30% of *S. elegans* and *S. setosa* with food in the southern North Sea, and Rakusa-Suszczewski (1969) who found 14.8–25.1% of *S. elegans* in the central North Sea with food.

It is possible that the causes of these differences between the reactions of *Spadella* and *Sagitta* are environmental, and that as *Spadella* normally lives on contact with the substratum the experimental conditions could be regarded as fairly normal although this would not be so for *Sagitta*. Although this would not explain Reeve's success, it is in keeping with Parry's remarks (1944) that *Sagitta* rarely feeds under experimental conditions. Some experiments were therefore carried out in 5-litre tanks but these were unsatisfactory, partly as there was no means of knowing the precise behaviour of the vibrating probe with varying lengths below the surface, but mostly as the speed of the *Sagitta* and their great transparency made their movements impossible to observe with precision. Indeed after sudden darts they would be lost to view for several minutes.

Parry also states that *Sagitta* only feeds under dim light or in the dark. To observe their reactions they had to be viewed with some light, and for microscopic observation the light was necessarily moderately bright, but the indoor daylight used for the dish and tank experiments was subdued.

Further research on stimulating *Sagitta* to feed by this method was therefore abandoned.

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Seasonal Changes in the Limnology of Some Meromictic Lakes in Southern British Columbia

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Seasonal changes in physical, chemical, and biological features are described in four small lakes which show different types and degrees of meromixis. There is a wide range in total dissolved solid content between lakes as well as within lakes (surface to near-bottom): Yellow, 282 (surface) to 337 (bottom); White, 6229–7918; Mahoney, 10,003–86,906; Lyons, 12,116–222,195 mg/liter, but no marked seasonal changes. In Yellow Lake meromixis is probably maintained by morphometric features of the lake basin rather than a strong chemocline; in the other lakes complete mixing is inhibited by chemical density gradients, those in Mahoney and Lyons being especially sharp.

Summer, autumn, winter (under ice), and spring vertical profiles are presented for temperature, dissolved oxygen, *p*H, alkalinity, specific conductivity; differences within and between lakes are discussed. Other physical–chemical characteristics are given in less detail.

Purple sulphur bacteria occur in three of the lakes (White, Mahoney, Lyons) and in the latter two form discrete "plates" detectable by high frequency echo sounding (confirmed by sampling). Seasonal changes in depth of the bacterial plates are considered in relation to some physical and chemical parameters. Phytoplankton, zooplankton, and benthos communities are discussed in relation to the degree of meromixis shown by the lakes.

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INTRODUCTION

In a region such as British Columbia which exhibits considerable limnological diversity (Northcote and Larkin, 1963), it is surprising that inland meromictic lakes have not been described previously, although several coastal meromictic lakes are known (Williams et al., 1961; Northcote et al., 1964; Northcote and Johnson, 1964).

The commonly accepted definition of meromixis — a lake in which some water remains partly or wholly unmixed with the main water mass at the circulation periods (Hutchinson, 1957) — does not preclude the possibility that mixing may be inhibited by means other than chemical density gradients, but this is usually implied, especially since the boundary between the mixolimnion and the monimolimnion is designated as a "chemocline." However,

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in some lakes meromixis probably results initially from morphometric characteristics of lake basins rather than from chemical stratification.

Such a condition is reported herein for one British Columbia lake and compared with three other lakes where meromixis is chemically induced. Seasonal changes in physical, chemical, and biological characteristics of the lakes are described and compared. The data were obtained in the course of a general survey of several lakes where rainbow trout populations could not be maintained by stocking.

METHODS

Contour maps of all lakes were based on echo-sounder transects applied to outlines made from suitable maps and low level air photographs. Temperature series were taken with a thermistor (Y.S.I. Model 44TB). Light penetration was measured with a standard Secchi disc using a viewing box; two series were made with a photometer (GM Model 15-M-02/1).

Most dissolved oxygen determinations were made with unmodified Winkler analysis from samples collected with a 1-liter Kemmerer bottle or a 2-liter van Doren bottle; a recently calibrated F.B.A. Model oxygen probe was used in the 1968 series. In situ conductivity readings were made with a model RB2-3341 Solu Bridge (Industrial Instruments Inc.); some readings were taken on water samples in the laboratory with an Industrial Instrument RC-7 conductivity meter; all values were calibrated with the latter instrument at 25 C. An Analytical Pocket *p*H meter was used in 1961; in 1968, a Radiometer Type PHM 24e meter. Alkalinity and total dissolved solid determinations (by evaporation) followed methods outlined in Standard Methods (American Public Health Association, 1960), using 0.1 N H_2SO_4 for alkalinity titrations. Analysis for dissolved H_2S followed the method given by van Gemerden (MS, 1967 and personal communications) using a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer at 668 mμ; because of incomplete precipitation at higher H_2S concentrations, values given are probably conservative. Specific ion analyses were made according to Standard Methods (American Public Health Association, 1965) by Wood Laboratory, Vancouver, B.C.

Echo tracings of bacterial plates were made with a Furuno F-701 200 Kc/sec sounder. A sample of purple bacteria taken from Mahoney Lake in November 1967 was subject to spectral analysis. One of four cell pellets obtained by centrifuging was extracted with pyridine, dried with N_2, dissolved in petroleum ether (bp 38–52 C) and applied to a chromatograph column of Al $(OH)_3$. A green pigment (presumably a bacteriochlorophyll) and a red-purple pigment were separated by the column. Only the red–purple pigment could be eluted from the column, and this was done with petroleum ether and 2.5% acetone. The spectra reported here were read in petroleum ether and CS_2. Zooplankton samples were collected from a total vertical water column with a standard (25 cm D) Wisconsin #10 net (0.158 mm mesh) at a central station in each lake (Fig. 1). In 1966 a Clarke–Bumpus sampler (#10 net) was used in Mahoney Lake. Twenty-four-hour settled volumes of zooplankton samples were converted to dry weight (mg/m^3) using a conversion factor drawn from a graphical correlation of settled volume with dry weight. A 15-cm Ekman dredge was used to take benthos samples, which were washed through a 1-mm mesh screen.

FORMATION AND MORPHOMETRY OF THE LAKE BASINS

The four lakes lie in the Southern Interior Plateau limnological region (Northcote and Larkin, 1956, 1963). Locations and morphometry of the lake basins are shown in Fig. 1 and Table 1.

Yellow Lake basin was cut into Tertiary volcanic rocks (mainly basalt and andesite) by a major meltwater outflow which drained a large ice lobe

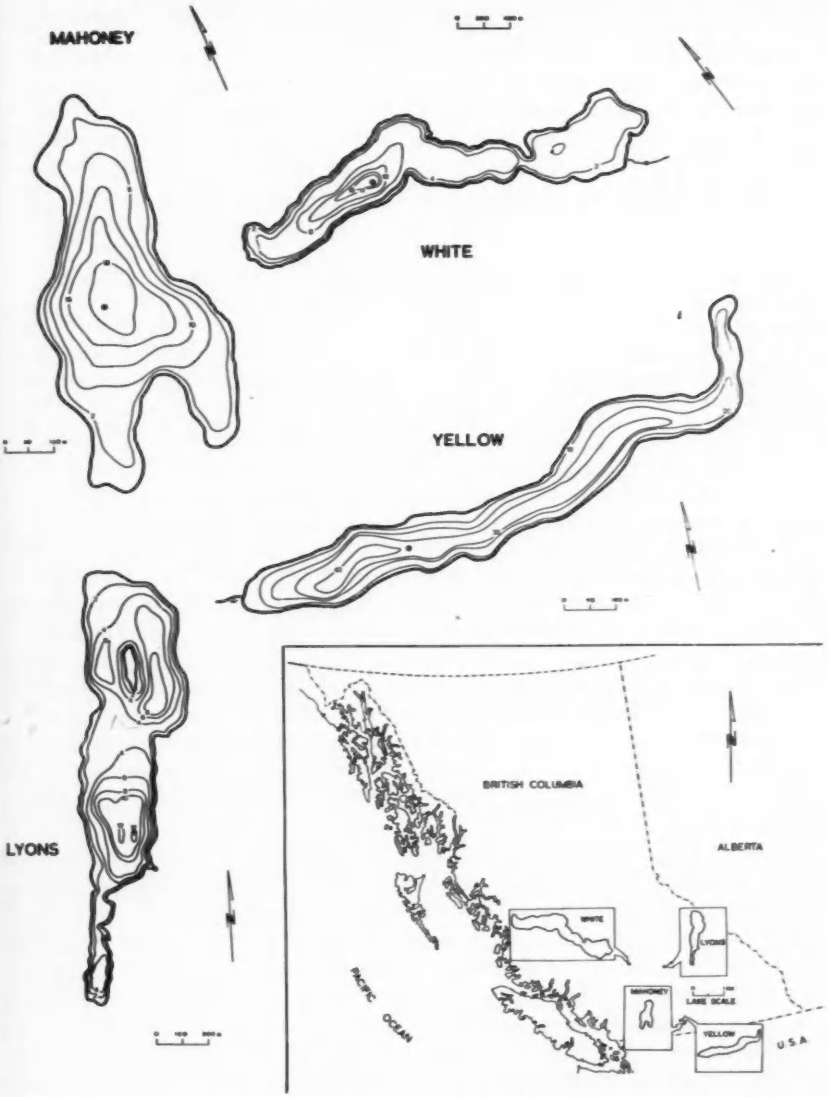
MAHONEY
WHITE
YELLOW
LYONS
BRITISH COLUMBIA
ALBERTA
PACIFIC OCEAN
WHITE
LYONS
MAHONEY
YELLOW
LAKE SCALE
U.S.A.

FIG. 1. Location and morphometry of four meromictic lakes in British Columbia. Depth contours in meters.

in the Okanagan and the Similkameen valleys (Cairnes, 1940; Nasmith, 1962). The Yellow Lake channel was utilized during at least two stages in recession of the Okanagan ice lobe, which may account for the steep-sided deep basin occupied by the lake now.

TABLE 1. Morphometric and some geographic features of four meromictic lakes[a] in southern British Columbia.

Characteristic	Yellow	White	Mahoney	Lyons
Elevation (*m*)	750	1,037	472	686
Max level change (*cm*) (high spring 1961–low spring 1962)	74.9	53.3	74.9	8.9
Drainage area (*ha*)	1,872	10,221	145	648
Surface area (*ha*)	32.3	99.6	21.6	35.7
Length (*m*)	1,851	2,430	895	1,580
Max depth (*m*)	40.0	15.0	18.9	12.0
Mean depth (*m*)	20.45	4.62	7.16	4.80
Mean/max depth (*m*)	0.51	0.40	0.38	0.40
Volume ($m^3 \times 10^4$)	660.1	460.2	154.9	171.3
Max depth of mixolimnion (*m*)	10	7	8	5
Mixolimnion as % of lake volume	45.0	90.7	68.6	78.8

[a]As defined by Hutchinson (1957).

Although no outcrops of bedrock are apparent near White Lake itself, sedimentary and igneous rocks as well as flat-lying beds of Tertiary basalt are evident in the general area (Reinecke, 1920). The basin lies in the line of several meltwater channels and may have been joined with them during glacial recession. A very large hydromagnesite ($4MgO \cdot 3CO_2 \cdot 4H_2O$) deposit is located along the north side of a tributary to White Lake (Reinecke, 1920).

Mahoney Lake lies astride a north–south oriented fault between Tertiary volcanic (the same series as at Yellow Lake) and granitic gneiss of the Shuswap Complex (Cairnes, 1940). Its present basin is adjacent to several regions showing outwash terraces or kettled outwash, and probably represents a deep kettle.

Lyons Lake is underlain by Palaeozoic sedimentaries and igneous rocks of the extensive Cache Creek group. This formation is rich in soluble carbonate minerals, and Dawson (1895) refers to a limestone outcrop near Lyons Lake. Along the gentle slopes forming the eastern side of the lake valley are a series of distinct, closely spaced shorelines representing previous glacial lake levels, the highest of which is at about 731 m (Dawson, 1895), some 45 m above the present level of Lyons Lake. Apparently retreat of the ice front on the western side of the valley was slowed considerably (Mathews, 1944), so that the series of lakes occupying it were relatively long-lived and may have received drainage from other glacial lakes in the Louis Creek valley to the east.

Except for White Lake, which covers nearly 100 ha, the lakes are small in both surface and drainage area (Table 1). Yellow Lake occupies a narrow, deep trough as indicated by its high $\bar{Z}$:Zm ratio, typical of overdeepened valleys (Hutchinson, 1957); ratios for the other basins are well within the usual range for readily eroded glacial drift. Although the maximum observed depth of the mixolimnion is considerably greater in Yellow than in the other lakes, its mixolimnion contributes less than 50% of the total volume of the lake. This feature is further emphasized when one notes that Yellow has the second smallest surface area but the largest volume of the four lakes. All lakes except Lyons drop between a half to three-quarters of a meter in surface level between spring and winter; seasonal level fluctuation is minimized in Lyons by surface and perhaps subsurface springs. Its level has risen at least 1 m in the last few decades.

PHYSICAL–CHEMICAL CHARACTERISTICS

TEMPERATURE

Thermal profiles of Yellow and White lakes were not strongly indicative of meromixis (Fig. 2). Both showed a typical "eutrophic" pattern of summer thermal stratification, with hypolimnial temperatures near 4 C. Winter profiles of these two lakes indicated an inverse curve under ice cover, but that for White was somewhat unusual, since the low temperature water (<2 C) extended to at least 8 m. Presumably White Lake continued to circulate throughout much of its volume until late autumn before its ice cover formed, and even thereafter cooled further (Fig. 2, February, April 1962 profiles).

On the other hand, vertical changes in temperature alone would suggest that Mahoney and Lyons lakes were markedly meromictic. Throughout the year, the monimolimnion remained about 16 C in Mahoney and 14 C in Lyons, and distinct maxima were evident in their chemoclines during autumn. Winter profiles indicated clearly the lack of circulation in deeper layers.

TRANSPARENCY

Secchi disc readings were much less in Yellow and White lakes than in the other lakes (Fig. 2), a result of heavy blue-green algal blooms in Yellow at least. In the more obviously meromictic lakes (Lyons and Mahoney), the mixolimnion was appreciably clearer, so that Secchi disc readings usually approached or exceeded 3 m. Photometric readings are given for Mahoney Lake in Fig. 7.

DISSOLVED OXYGEN

The vertical distribution of dissolved oxygen (Fig. 3) clearly showed the meromictic conditions prevailing in each of the lakes. Yellow was devoid of oxygen below 10 m throughout the year and during most of the summer and winter, *below 2 m*. All lakes except Mahoney showed severe winter stagnation in the mixolimnion, and values immediately beneath the ice often fell below 1 mg/liter. Mahoney and Lyons frequently had distinct maxima near the lower level of the mixolimnion, possibly a result of photosynthetic production from concentrated algal layers. Heavy concentrations of *Oedogonium* sp. and *Campylodiscus* sp. were found to coincide with the oxygen maximum in Mahoney Lake, September 1966 (Fig. 3, Table 4).

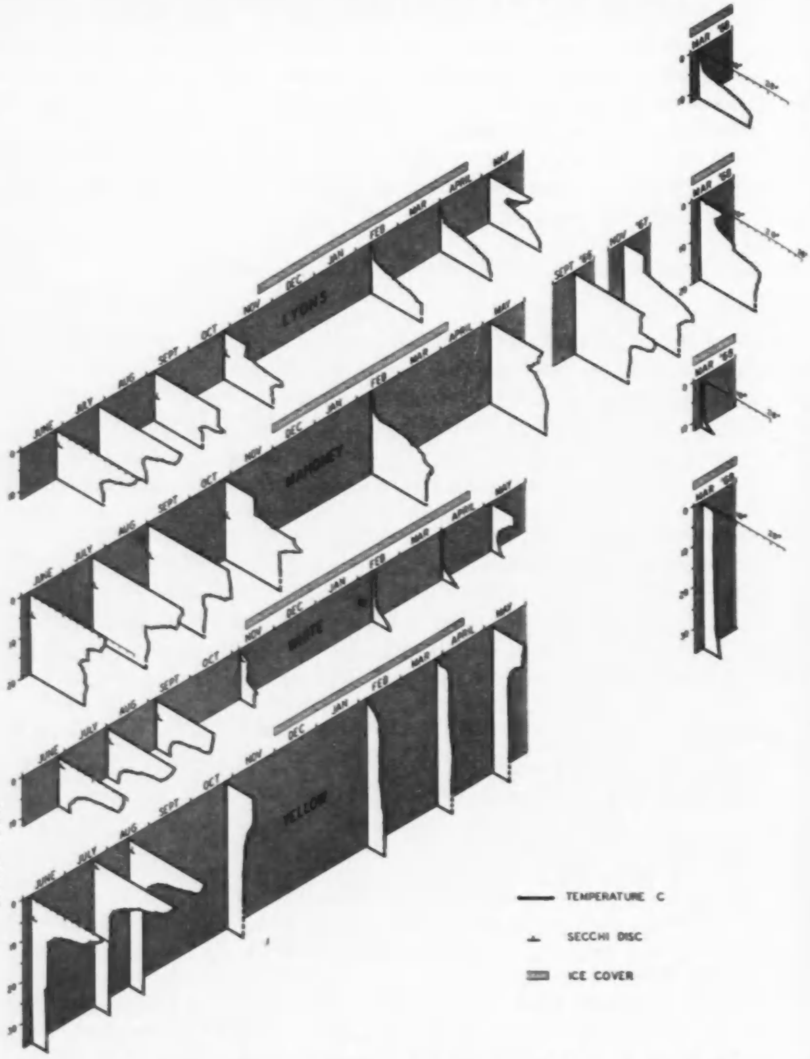

FIG. 2. Seasonal thermal profiles and light penetration in four meromictic lakes, June 1961 to May 1962, with some additional profiles in 1966–68.

pH, ALKALINITY, AND H_2S

All lakes had pH values over 7, those for Yellow being the lowest, especially in the deeper water (Fig. 4), where readings were at least a full unit below those near the surface. White Lake had a consistently high pH (ap-

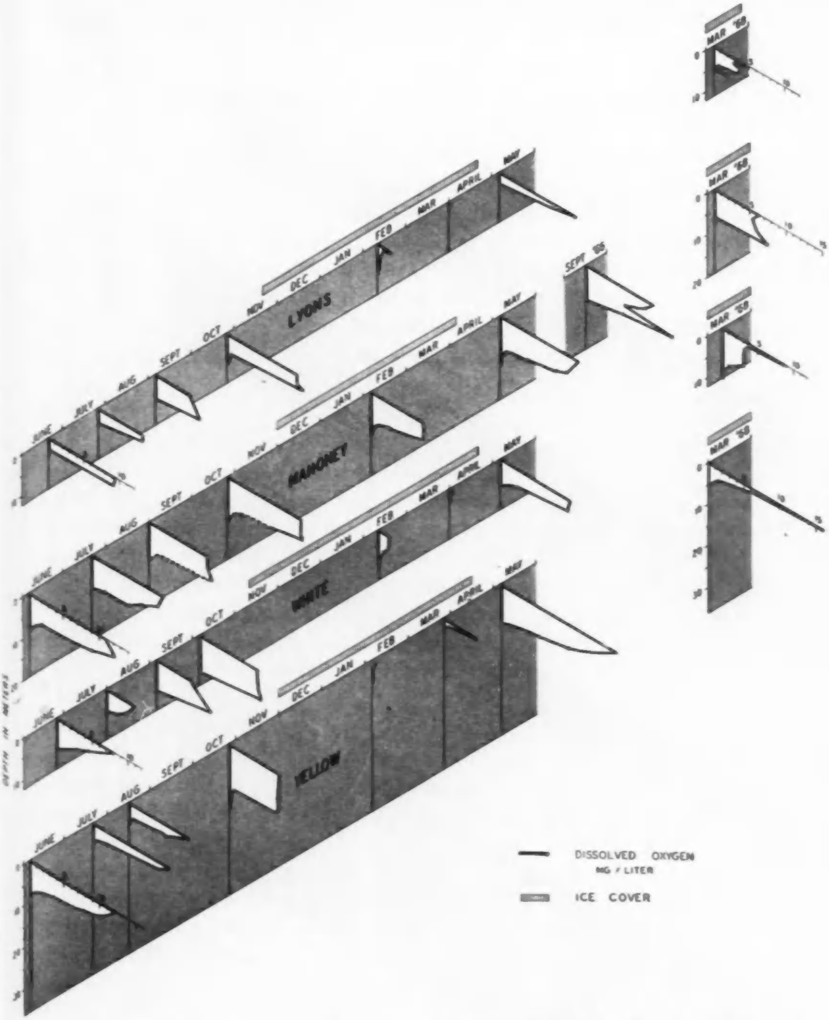

FIG. 3. Vertical distribution of dissolved oxygen in four meromictic lakes, June 1961 to May 1962, with some additional data in 1966 and 1968.

proaching 10) with little vertical or seasonal change. In Mahoney and Lyons, the *p*H was consistently higher in the mixolimnion than in the monimolimnion, occasionally with maxima in the lower mixolimnion.

The total alkalinity of Yellow Lake was much lower than that of the other lakes, usually between 250 and 300 mg/liter with minimal vertical

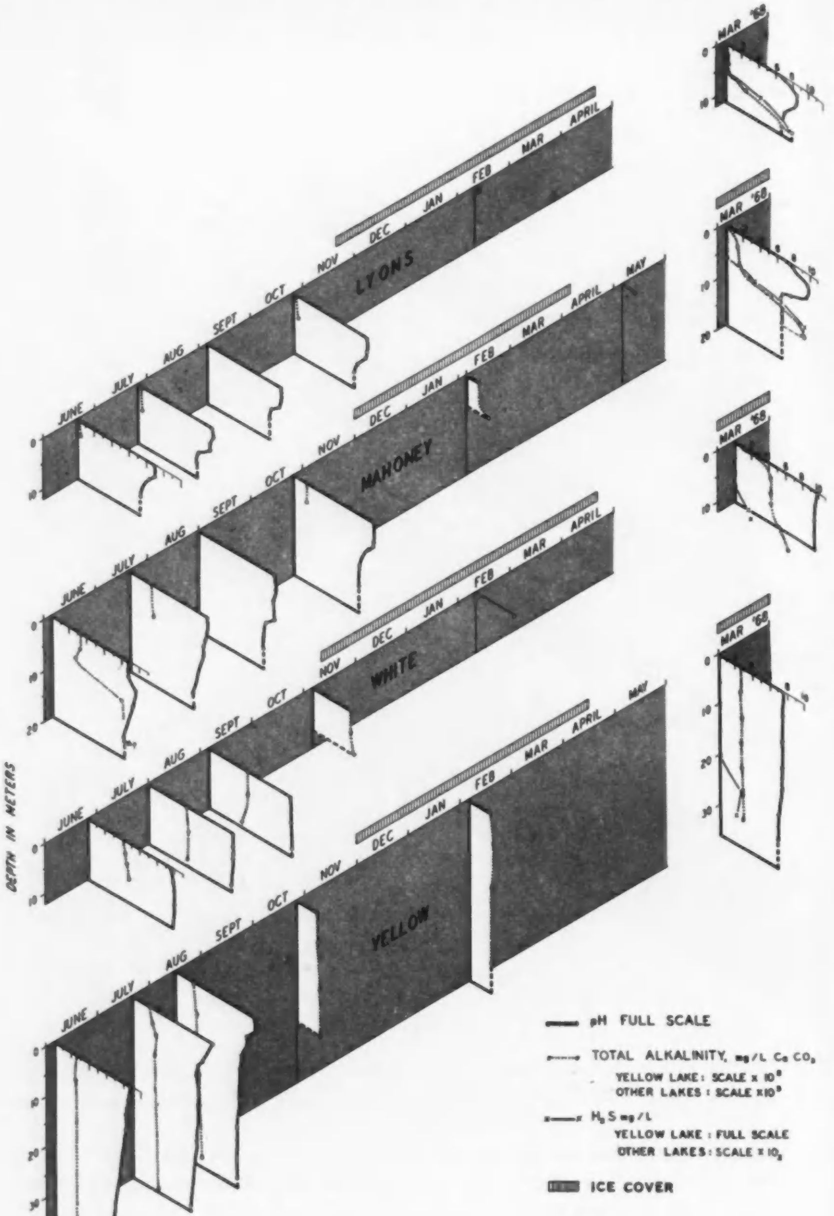

FIG. 4. *p*H and alkalinity profiles in four meromictic lakes, June 1961 to May 1962, with an additional series including H_2S in March 1968.

or seasonal changes (Fig. 4). White had the most alkaline mixolimnion of the four lakes, about 4500 mg/liter except for a low near-surface value in March 1968 (probably resulting from snow meltwater entering through ice cracks). Its monimolimnial water (5000–6000 mg/liter) was lower in alkalinity than Mahoney or Lyons (8000–9000 mg/liter).

Although an H_2S odour was noted in the near-bottom water samples of all lakes throughout the 1961–62 season, quantitative measurements were not made until March 1968 (Fig. 4). In Yellow Lake H_2S was not encountered until 25 m, and from there to 30 m values were slightly in excess of 2 mg/liter. Similarly in White Lake only the near-bottom water contained H_2S, although values were nearly 100 times those at Yellow. In Mahoney and Lyons lakes, H_2S appeared and sharply increased in the monimolimnion, with very high concentrations (800–900 mg/liter) near the bottom.

CONDUCTIVITY

Marked differences in conductivity profiles were evident between the four lakes (Fig. 5). In Yellow Lake there was only a very slight increase in conductivity from surface to near bottom water (most readings ranging from 400 to 500 μmhos), and little seasonal change except for a decrease immediately beneath the ice in winter. Conductivity of White Lake was more than 10 times higher than at Yellow, with a more obvious increase towards the bottom. In Mahoney and Lyons lakes the profiles clearly showed sharp increases between the mixolimnion and monimolimnion, with the latter being much higher in conductivity than in Yellow or White.

TOTAL DISSOLVED SOLIDS

Vertical and seasonal differences in total dissolved solids within and between lakes (Table 2) followed the pattern indicated above for conductivity. Dissolved solids in Yellow Lake were more than an order of magnitude lower than the other lakes, showed only a minor increase in bottom water, and fluctuated little seasonally. An obvious but not marked increase in dissolved solid content of near bottom water was evident in White Lake; winter values (both surface and near-bottom) were somewhat higher than those for other seasons. Surface dissolved solids in Mahoney and Lyons were about double those in White, whereas near-bottom determinations were at least 10 times higher, indicating a much greater density difference between mixolimnion and monimolimnion in these lakes than in White or Yellow. The low surface reading for Lyons in March was probably due to meltwater dilution.

MAJOR CATIONS AND ANIONS

Ionic compositions of mixolimnion and monimolimnion water in each of the lakes are given in Table 3. When converted to equivalent concentrations, major cations and anions balance reasonably well in Yellow and White lakes, but do not in Mahoney or Lyons. In these lakes difficulites in specific ion analysis at high concentrations may account for the discrepancies.

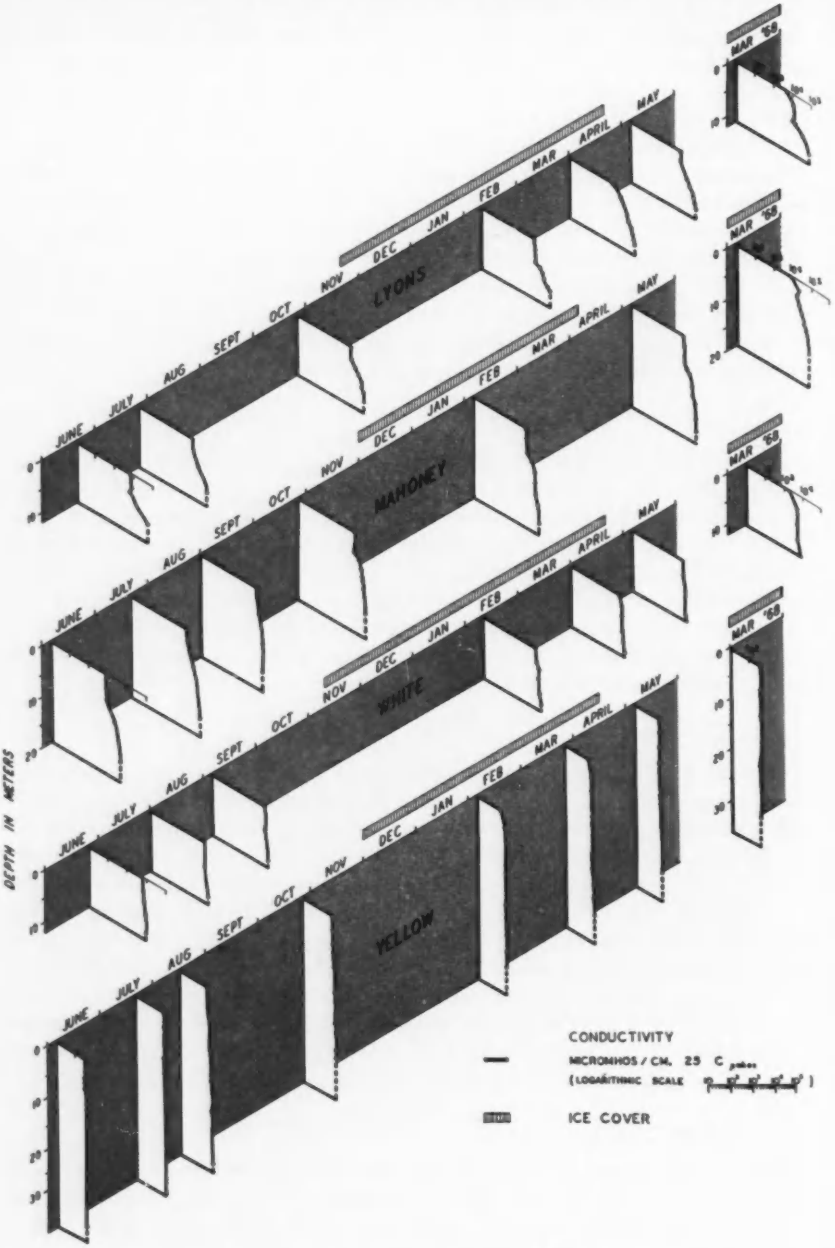

FIG. 5. Conductivity profiles in four meromictic lakes, June 1961 to May 1962, with an additional series in March 1968.

TABLE 2. Seasonal changes in total dissolved solid content (mg/liter) of surface and near-bottom water from June 1961 to May 1962.

Lake	Depth (*m*)	June	July	Aug.	Oct.	Feb.	Mar.	May	Avg
Lyons	Surface	12,088	13,098	13,092	13,702	12,704	8,010		12,116
	Near bottom 8–10 m	190,608	228,200	184,782	186,700	285,882	257,000		222,195
Mahoney	Surface	9,308	9,906	9,802	10,890	10,802		9,308	10,003
	Near bottom 15–18 m	83,104	87,598	87,824	88,004			87,998	86,906
White	Surface	5,714	6,094	6,504	6,014	6,700	7,086	5,490	6,229
	Near bottom 10–11 m	7,694	7,818	7,798		8,410	8,094	7,692	7,918
Yellow	Surface	298	250	271	321	349	203		282
	Near bottom 34–35 m	323	299	351		328	374	350	337

TABLE 3. Concentration (mg/liter) of major cations and anions in the mixolimnion and monimolimnion of the four meromictic lakes.

Lake	Date	Depth (*m*)	K^+	Na^+	Ca^{++}	Mg^{++}	Fe (*total*)	Mn (*total*)	$SO_4^=$	Cl^-	HCO_3^-	$CO_3^=$
Lyons	June 20, 1950	0	240	1,700	2,030	611	.05	–	2,980	7.9	–	–
	Mar. 6, 1968	4.5	305	1,336	2,932	1,509	.10	.07	8,738	111	320	5.5
	Mar. 6, 1968	7.0	410	6,165	14,879	2,978	.21	2.67	18,265	419	2,415	0
Mahoney	Mar. 4, 1968	5.0	650	3,949	2,226	1,234	.07	0	11,034	800	1,285	225
	Mar. 4, 1968	9.0	1,180	6,369	7,190	3,374	.17	.50	20,643	709	5,181	0
White	Mar. 7, 1968	3.0	287	1,211	221	209	.11	0	393	121	3,300	770
	Mar. 7, 1968	12.0	310	1,416	145	264	.11	.43	834	159	4,225	875
Yellow	Aug. 14, 1951	0	1.8	50.0	44.4	16.3	.01	–	7.9	5.1	–	–
	Feb. 29, 1968	5.0	0	64.2	32.4	0	.02	.14	0	9.4	250	0
	Feb. 29, 1968	30.0	0	62.5	66.4	0	.12	.36	0	7.1	280	0
Analysis method	Standard Methods (APHA, 1965)		Colorimetric	Gravimetric	Complexometric	Gravimetric	Phenanthroline	Persulfate	Gravimetric	Argentometric	Titration (*p*H meter)	

Marked differences were evident between lakes (Fig. 6). Lyons may be characterized as a "calcium–sulphate" lake, although Na^+ and Mg^{++} are also relatively high. Mahoney also is high in sulphates, but Na^+ and Mg^{++} are more important cations. On the other hand White is essentially a "sodium–bicarbonate" lake, whereas sodium and calcium predominate in Yellow. Slight differences are evident in ionic composition of the mixolimnia and monimolimnia of Lyons, Mahoney, and Yellow lakes.

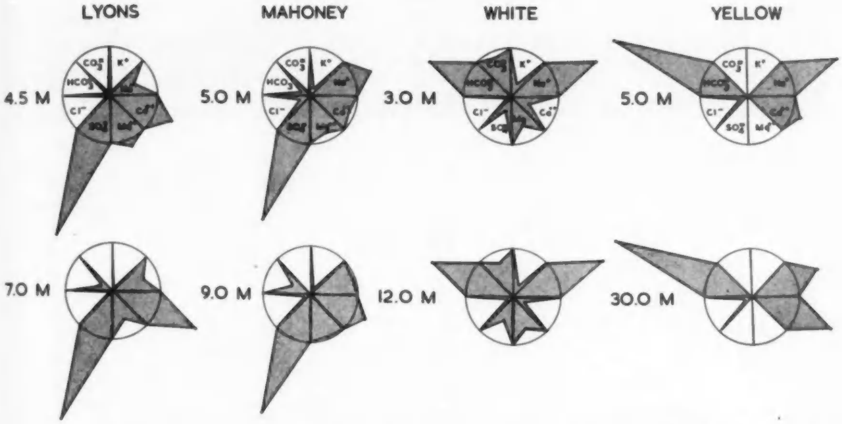

FIG. 6. Equivalent concentrations (per cent) of major anions and cations in the four meromictic lakes (following Hutchinson, 1957, fig. 155); concentrations of anions and cations balanced fairly closely for Yellow and White lakes, but anions were considerably below cation milliequivalents in Mahoney and Lyons lakes.

BIOLOGICAL CHARACTERISTICS

BACTERIAL PLATES

High frequency (200 kc/sec) echo soundings revealed distinctive midwater scattering layers in Mahoney and Lyons lakes during all seasons (Fig. 7 and 8) but not in either Yellow or White lakes. The layers were continuous horizontally across Mahoney Lake basin (Fig. 7, *top*) and Lyons Lake (not shown). In Mahoney Lake, two layers were recorded for all seasons except November 1967 and March 1968. The "upper" layer was not recorded during the latter periods possibly because of reduction in the sensitivity of the echo sounder or because of absence of the layer. The lower layer in Mahoney and the layer in Lyons was shown by Wisconsin net stage hauls and water bottle samples to be purple sulfur bacteria which were positioned on the chemocline. The predominant bacterium was identified as *Thiocapsa* sp. (1968 sample, Mahoney). Clarke–Bumpus samples taken in 1966 confirmed earlier data on the position and composition of the lower scattering layer and indicated that the upper

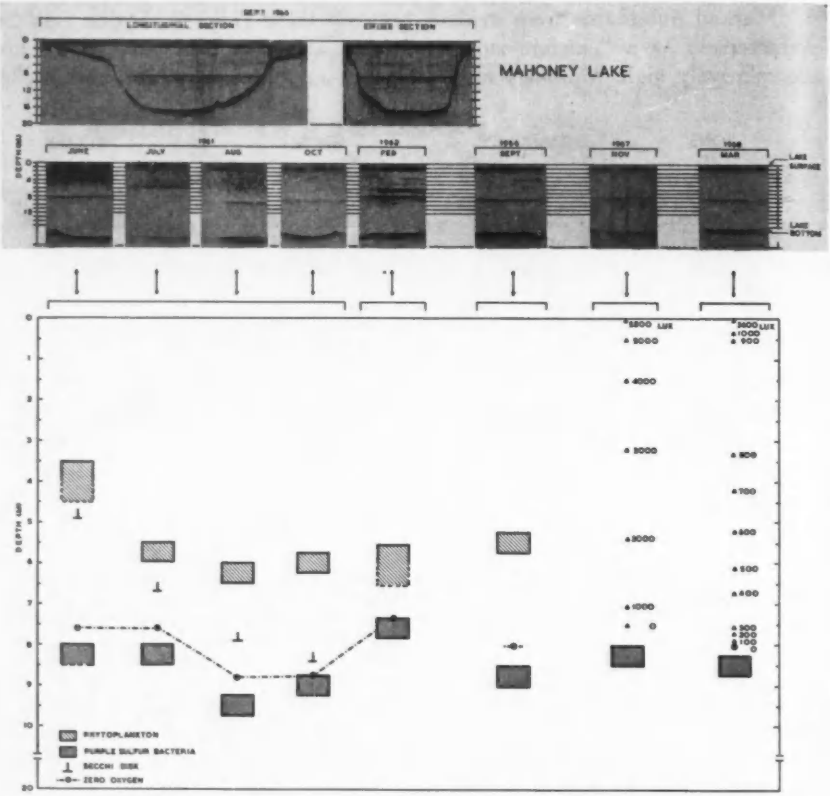

FIG. 7. Seasonal changes in the depth of the bacterial plate and a second scattering layer in Mahoney Lake. Note: the depth shown for "zero" oxygen is only approximate.

layer was probably caused by phytoplankton, largely *Oedogonium* sp. and *Campylodiscus* sp. Counts of the Clarke–Bumpus samples showed that maximal numbers of these organisms (Table 4) were coincident with the upper scattering layer.

Absorption spectra of the carotenoid pigment extracted from bacteria taken from about 9 m in Mahoney Lake in November 1967 showed absorption peaks of (454), 483, 515 mμ in petroleum ether (bp 38–52 C). These are very close to the absorption maxima shown for the carotenoid Hydroxy-P 481: 455, 483, 516 mμ in Light Petroleum (Davies, 1965). In CS_2 the sample had absorption maxima at (490), 522, 559 mμ, which were similar to those shown by Davies (1965) for Hydroxy-P 481 (491, 522, and 559 mμ) and Rhodovibrin (491, 522, and 559 mμ) in CS_2. Jensen (1963) shows the Rhodovibrin (OH-P 481) group of carotenoids to be present in the Athiorhodaceae (***Rhodospirillum photometricum***, ***R. rubrum***, ***Rhodopseudomonas palustris***, ***R. gelatinosa***), in

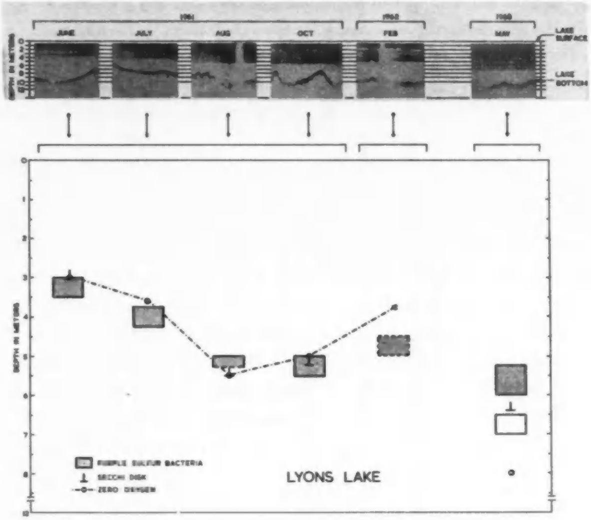

Fig. 8. Seasonal changes in the depth of the bacterial plate in Lyons Lake. Note: the depth shown for "zero" oxygenis only approximate.

the Thiorhodaceae (*Chromatium* sp. strain D), and in the Hyphomicrobiaceae (*Rhodomicrobium vaniellii*).

Seasonal changes in the level of the layers were evident in both lakes; the position of the bacterial plate increased in depth as light penetration and the depth of the mixolimnion increased (Fig. 7 and 8, *bottom*). Underwater photometer readings indicated that the bacterial plate was positioned just under the "zero" lux level (Fig. 7, November 1967, March 1968). Absolute thicknesses of the scattering layers were not easily compared between seasons because the echo sounder was not always operated at the same sensitivity.

PHYTOPLANKTON

Aphanizomenon sp. was present in most samples collected from Yellow Lake, but was relatively rare in spring of 1961, when *Nostoc* sp. was abundant. Heavy algal blooms were evident there in summer when *Aphanizomenon* sp., *Anabaena* sp., and *Nostoc* sp. were dominant. Blooms similar to those observed in Yellow Lake were not observed in the other meromictic lakes. Concentrations of *Oedogonium* sp. and *Campylodiscus* sp. in Mahoney Lake are noted above. A layer of phytoplankton may have caused a second scattering layer at 6 m in Lyons Lake in May 1968 (Fig. 8).

ZOOPLANKTON

Five different species of *Diaptomus* and one *Cyclops* sp. as well as three species of cladocerans were found in the lakes (Table 5). Standing crops of

TABLE 4. Estimates of zooplankton and phytoplankton abundance (number/ml) in preserved Clarke–Bumpus samples, Mahoney Lake, September 14, 1966 (based on averages of four 1-ml subsamples, counting 4 of 24 divisions in a Sedgwick–Rafter cell). *Oedogonium* counts indicate number of filaments.

Depth (*m*)	*Diaptomus connexus*	*Ceriodaphnia lacustris*	*Brachionus plicatilus*	*Keratella* sp.	Copepodites	*Campylodiscus* sp.	*Oedogonium* sp.
2.00	138	46	9	60	9	31	51
5.25	100	0	15	73	6	180	261
6.50	73	1	33	40	0	108	174
8.75	0	0	0	0	0	0	0
9.50	1	0	0	0	0	0	141

TABLE 5. Copepods and cladocerans identified in the four meromictic lakes.

Yellow	White	Mahoney	Lyons
Diaptomus leptopus	*Diaptomus nevadensis*	*Diaptomus tyrelli*	*Diaptomus sicilis*
Cyclops sp.	*D. sicilis*	*D. connexus*	*Daphnia pulex*
Daphnia schodleri	*Daphnia schodleri*	*Ceriodaphnia lacustris*	

zooplankton decreased in all four lakes from early summer to autumn (Table 6). Some fluctuation was evident in Lyons during this period, but the autumn value was nonetheless lower than that of early summer. Zooplankton abundance was generally lower in spring and winter than in summer for all lakes. Usually summer and autumn standing crops of zooplankton were highest in Yellow Lake (the least saline of the series), lower in White and Mahoney, but higher in Lyons (the most saline of the four lakes). Differences in standing crop between lakes in winter and spring were small.

TABLE 6. Standing crop (mg/m^3, dry wt) of crustacean zooplankton in the mixolimnia of the four meromictic lakes.

	1961				1962		1968
Lake	June	July	Aug.	Oct.	Feb.	May	Mar.
Yellow	83	48	39	29		10	8
White	34	34	22		22	19	13
Mahoney	24	17	10	9	16	15	16
Lyons	59	34	43	22	30		11

Diaptomus connexus and *Brachionus plicatilis* in Mahoney Lake had yellow-stained mouth parts and anus (*D. connexus* only) during all seasons. In Lyons Lake *B. plicatilis* was similarly stained in the autumn. This suggests that these zooplankters fed on sulfur bacteria.

Chaoborus larvae were common (ca 10–20/m^3) throughout all seasons in Lyons Lake. They also occurred in the other lakes but were not so frequently taken.

BOTTOM FAUNA

Although the benthic organisms in the meromictic lakes were not well sampled, the abundance and depth distribution of common forms can be outlined (Fig. 9, Table 7) As might be expected, numbers declined rapidly between the mixolimnion and the monimolimnion in each lake — a few *Chaoborus* or chironomid larvae being the only forms commonly found near the boundary. Amphipods, damselfly nymphs, and chironomid larvae accounted for the majority of the benthos in the mixolimnetic region.

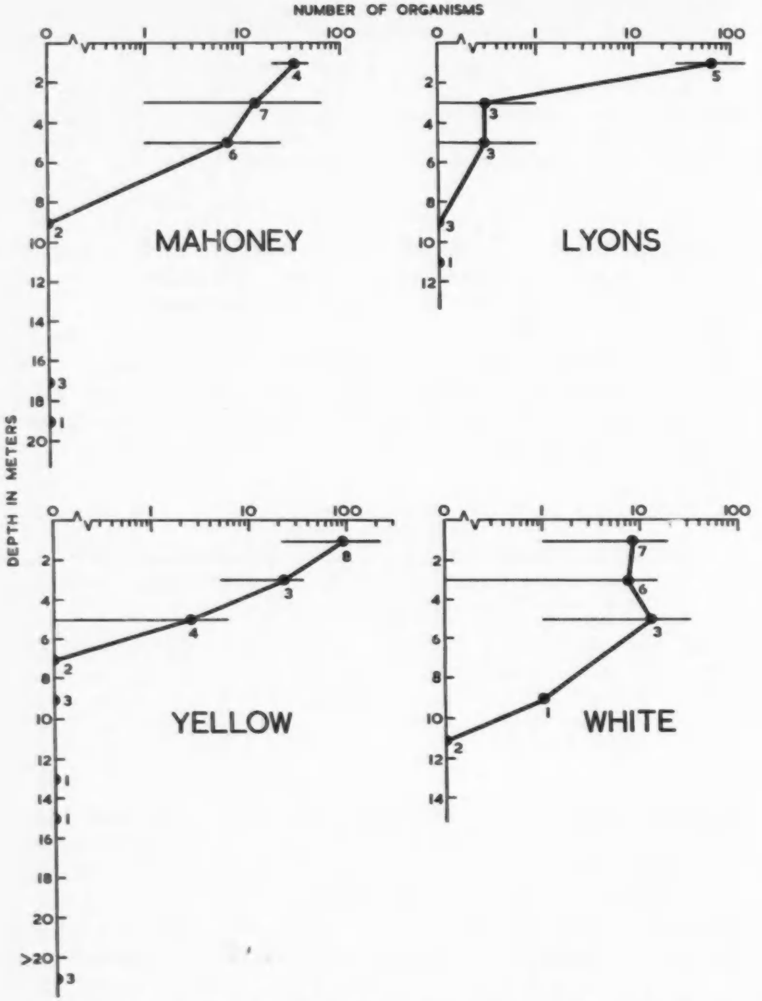

FIG. 9. Vertical distribution of bottom fauna (total number/225 cm^2) in the four meromictic lakes in summer (1961). Horizontal lines indicate ranges; number of samples given beside points for each 2-m depth interval.

DISCUSSION

The four lakes in our study made an interesting series in which to consider causes and origins of meromixis. Yellow Lake, although highly meromictic in that only a small part of the water in its basin freely circulated each year, showed very little evidence of mixing being inhibited by a chemical density gradient. The cause of meromixis in Yellow Lake would seem to be largely, if not entirely, morphometric; it lies in a very deep, narrow basin and is protected by local topography from strong wind action except occasionally in the direction of its long axis. Although the increase in dissolved mineral content of its bottom water has probably been a result of biogenic processes, it does not seem reasonable to suggest these as the cause of meromixis since this increase occurred at a level far below that of the "boundary" between the mixolimnion and monimolimnion. Other workers have noted that meromixis develops in lakes with deep basins sheltered from prevailing winds — Likens (MS, 1967) for Lake Mary, Wisconsin; Likens (MS, 1962, MS 1967) for Stuart's Dark Lake, Wisconsin; Edmondson (1963) for Hall Lake, Washington — but the origin and maintenance of this condition is usually attributed to biogenic processes. For a lake such as Yellow, morphometric features alone may be sufficient to induce meromixis and the gradual buildup of chemically denser water in the lower level of the monimolimnion would then be a result rather than a cause of meromixis. In this sense it would seem appropriate to recognize a fourth type — ***morphogenic meromixis*** — in addition to the three caterogies (ectogenic, crenogenic, and biogenic) suggested by Hutchinson (1957). Morphogenic meromixis would then seem to include instances such as Lake Baikal, where extreme depth of the lake restricts mixing — "meromicticité mécanique" (Dussart, 1966) and other conditions described by Olszewski (1953).

In contrast, White Lake, the largest in area and second shallowest of the four lakes, is probably an example of biogenic meromixis, although the possibility of mineral-laden spring water entering its bottom water from surrounding deposits should not be discounted. Mahoney and Lyons lakes probably illustrate situations where meromixis has been maintained by biogenic processes.

Edmondson (1963) has tabulated chemical characteristics for meromictic lakes in the western United States, and Likens (MS, 1967) in expanding coverage to North America has attempted to draw relationships between ionic ratios or dominant ions and the three types of meromixis recognized by Hutchinson (1957). Thus lakes of ectogenic–crenogenic origin had low Ca/Mg ratios, high Na/K ratios, with chloride and sulphates the dominant anions; biogenic lakes had high Ca/Mg and low Na/K ratios with bicarbonates the major anion. This relationship did not seem to hold in our lakes, since in those of probable biogenic origin (Lyons, Mahoney, and White) Na/K ratios were always much higher than those for Ca/Mg and sulphates were the dominant anion in at least two (Table 3).

Seasonal changes in physical–chemical conditions in the one morphogenic lake (Yellow) were not strikingly different from those in typical eutrophic lakes, except for complete and year-round deoxygenation of the lower layers (20–33 m). In the other lakes of biogenic origin, many of the characteristics associated with meromixis were evident during most seasons. Thus abnormal thermal profiles were usually evident, and two of the lakes, Mahoney and Lyons, had persistently high monimolimnial temperatures, over 16 and 13 C respectively. Of the several mechanisms suggested for maintaining warm

TABLE 7. Bottom fauna in different depth zones of the four meromictic lakes during summer.

Depth zone (*m*)	Yellow	White	Mahoney	Lyons
0–2	Demospongiae			
	Spongillidae			
	Hydrozoa			
	Hydra sp.			
	Turbellaria			
	Bryozoa			
	Oligochaeta			Oligochaeta
	Hirudinea			
	Crustacea		Crustacea	Crustacea
	Gammarus lacustris			
	Hyalella azteca		*Hyalella azteca*	*Hyalella azteca*
	Insecta	Insecta	Insecta	Insecta
	Ephemeroptera	*Enallagma* sp. (nymph)	*Enallagma* sp. (nymph)	*Enallagma* sp. (nymph)
	Procladius (s.s.)	*Procladius* (s.s.)	*Tanypus*	*Chironomus*
	Procladius (*Psilotanypus*)	*Cenocorixa bifida*	*Chironomus* (s.s.)	*Tanytarsus*
	Glyptotendipes (*Phytotendipes*)	*Deronectes spenceri*	*Cryptochironomus*	
	Tanytarsus	Trichoptera	*Tanytarsus*	
	Arachnida		*Notonecta kirbyi*	*Notonecta kirbyi*
	Acarina		*Cenocorixa bifida*	*Haliplus strigatus*
	Gastropoda		*Cenocorixa expleta*	
	Planorbidae		Arachnida	
			Acarina	
	Turbellaria			
	Hirudinea			
	Crustacea	Crustacea		
	Gammarus lacustris	*Branchinecta mackini*		
	Hyalella azteca			

2–4		Insecta Ephemeroptera Tabanidae *Procladius* (s.s.) *Chironomus* (s.s.)	Insecta Anisoptera Coleoptera (adult) *Enallagma* sp. (nymph) *Chironomus* (s.s.)	Insecta Chironomid larvae *Chaoborus* larvae
4–6	Oligochaeta Crustacea *Gammarus lacustris* *Hyalella azteca* Arachnida Acarina Gastropoda *Physa* sp.	Insecta *Chironomus* (s.s.)	Insecta Anisoptera *Chironomus* (s.s.) Psectrocladius	Insecta Chironomid larvae
6–8	Nil	Insecta Chironomid larvae	Nil	Nil
8–10	Nil	Insecta Chironomid larvae	Nil	Nil
>10	Nil	Nil	Nil	Nil

monimolimnia (Hutchinson, 1957), none singly would seem to explain those observed here.

The chemocline of meromictic lakes provides the necessary H_2S and CO_2 which enable massive concentrations of purple sulphur bacteria to develop in "plates." Although the location of bacterial plates in meromictic lakes is well documented in the literature (Frey, MS, 1967; Truper and Genovese, 1968; Takahashi and Ichimura, 1968), seasonal changes in the vertical position of bacterial plates are not. Sorokin (1966, Fig. 3) showed a diel shift in maximum numbers of purple bacteria from 10 m at 0500 hr to 12 m at 1300 hr. Similarly Sorokin (1966, table VI) showed that there were three times as many purple sulphur bacteria at 10 m on an overcast day as there were on a clear day. Englemann in 1883 (Stephenson, 1939) showed that photosynthetic bacteria on a glass slide collected in the regions of their own absorption bands and that irradiation conditioned motility. Increases in the depth of the bacterial plates in Mahoney and Lyons lakes were associated with increases in light penetration (Fig. 7 and 8, June–August) and decreases in depth of the plates were associated with decreases in light penetration (August–February, Fig. 7 and 8). The magnitude of the changes (1.25 m in Mahoney and 2 m in Lyons) were similar to that shown by Sorokin (1966). No doubt the magnitude of vertical shift of the "plate" will be limited by availability of light, H_2S and CO_2 — and thus, the plate may shift downwards into the monimolimnion farther than it may move upwards from the chemocline. However, the shift in position may only be apparent and in fact be a change in species number or composition. The changes in position of the plates demonstrated in this study may have been a response to increases in oxygen concentration of the lower mixolimnion (Fig. 7 and 8), but responses of purple sulphur bacteria to light seem to suggest that it is the primary controlling factor. That these bacteria respond to light had been demonstrated by Cohen-Bazire (1963) who showed a five-fold reduction in chlorophyll content of *Rhodospirillum rubrum* cultured at high light intensities (about 64,000 lux). Some purple sulphur bacteria are motile (i.e. *Chromatium*, van Niel, 1963) and are presumably able to respond to changes in the environment. For those that are nonmotile, a possible means of transport in a water column is suggested from the work of Newton (1963) who states that "one might expect light induced electron flow to alter the mechanical properties of the cytoplasmic membrane and its permeability." Such changes may affect bouyancy of a cell.

The upper scattering layer of Fig. 7 coincided with a metalimnetic oxygen maximum on November 1966 (Fig. 3). Kuznetsov (1958, fig. 7) showed a similar maximum at 5 m in meromictic Lake Belevod but did not account for it. Stewart et al. (1966) attributed a metalimnectic oxygen maximum to *Cyclotella*, a diatom similar to *Campylodiscus* (the species concentrated in the upper scattering layer of Mahoney Lake, Table 4). Hopkins (1966) demonstrated that the diatom *Surirella gemma* migrated upward in a water column until suitable conditions for photosynthesis were met. *Campylodiscus* sp. and *Oedogonium* sp. may be positioned in a discrete layer in the water column to meet their photosynthetic needs. Several mechanisms to explain how phytoplankton may be maintained at a particular level in the water column are discussed by Gessner (1955).

Unlike the meromictic coulee lakes of Edmondson (1963), dense phytoplankton blooms were not recorded in White, Mahoney, or Lyons lakes. Paucity of phytoplankton was recorded for several meromictic lakes by Frey (MS, 1967). The occurrence of *Oedogonium* sp. at 9.5 m in Mahoney Lake (Table 4) is not easily explained. The filaments may have been dead at the time of collection, as it was unlikely that sufficient light for photosynthesis would have penetrated to that depth. Frey (MS, 1967) suggested that some blue-green algae are capable of utilizing H_2S. Although not recorded in Mahoney Lake, a narrow band of dissolved oxygen reappeared in Lyons Lake immediately beneath a bacterial plate above which dissolved oxygen was "zero" (Fig. 3, 8, 1968). Such a phenomenon could possibly be caused by H_2S-resistant phytoplankton photosynthesizing under a bacterial plate.

Sorokin (1965, 1966) has shown that maximum numbers of cladocerans and copepods occur during the day in the lower mixolimnion near the bacterial plate, whereas rotifers were distributed higher in the mixolimnion. Zooplankters were also found in the bacterial zone. The opposite distribution was found in Mahoney Lake midday samples where *Ceriodaphnia lacustris*, *Diaptomus connexus*, and *Keratella* sp. were in the upper mixolimnion but *Brachionus plicatilis* was most abundant near the bacterial plate (Table 4). Although Anderson (1958) stated that *Branchinecta* and *Artemia* were excluded from the bacterial zone by anaerobic conditions, Sorokin (1959, and subsequent papers) has conclusively demonstrated that *Daphnia* and *Diaptomus* as well as other plankters feed on purple sulphur bacteria. The yellow-stained mouth and anus of *D. connexus* at several levels in Mahoney Lake (Table 4) may suggest that these copepods migrate to and feed on the purple sulphur bacteria. Rotifers and cladocerans, in the meromictic lakes Soap and Lenore, were readily seen from the shore (Edmondson, 1963). Similar densities of cladocerans and copepods have been observed in Mahoney and Lyons lakes. Constant high temperatures in the monimolimnion of Mahoney and Lyons lakes could enable zooplankton to reproduce year-round (Anderson, 1958; Sorokin, 1966).

The vertical distribution of the benthos in the four meromictic lakes appears similar to that in severely stratified lakes noted by Frey (MS, 1967), where virtually no live organisms occurred in the monimolimnial layer. *Chaoborus* and chironomid larvae inhabited the boundary regions as found by Findenegg (1953, 1955, cited in Frey, MS, 1967) in the Carinthian meromictic lakes. In the region of the mixolimnion, a diverse and moderately abundant fauna was evident in Yellow, the least saline of the lakes, whereas in the others, numbers were generally lower and restricted to a shallow littoral zone, especially in Lyons.

Although attempts had been made to introduce rainbow trout into three of the lakes lacking fish (Yellow, Mahoney, and Lyons) these have all failed, probably because of low winter oxygen concentrations or high salinities.

Lake chub (*Couesius plumbeus*) have been seen in White lake, but only in the mouth of the inlet stream from Meadow Lake and in a small tributary (G. G. E. Scudder, personal communication). In Yellow Lake it might be possible to maintain a trout population by artificial autumnal circulation in a manner similar to that shown in another interior lake by Halsey (1968).

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Cortisol Secretion Rate in Gonadectomized Female Sockeye Salmon (*Oncorhynchus nerka*): Effects of Estrogen and Cortisol Treatment

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DONALDSON, EDWARD M., AND ULF H. M. FAGERLUND. 1969. Cortisol secretion rate in gonadectomized female sockeye salmon (*Oncorhynchus nerka*): effects of estrogen and cortisol treatment. J. Fish. Res. Bd. Canada 26: 1789–1799.

Gonadectomized female sockeye salmon (*Oncorhynchus nerka*) were injected intramuscularly with cortisol, estradiol, estradiol cypionate, or peanut oil for 8 weeks. The metabolic clearance rate of cortisol was determined by the single injection technique and calculated from the formula for a two pool system. The cortisol secretion rate was determined from the metabolic clearance rate and the plasma cortisol concentration.

The volume of distribution of cortisol in the inner compartment (V_1) and the outer compartment (V_2), the metabolic clearance rate (MCR), the resting secretion rate (S), and the resting plasma cortisol concentration were all significantly higher in the estradiol-injected group than in the peanut oil-injected control group, and the plasma cortisol concentration at the end of the experiment was lower. V_1, MCR, and S were also significantly higher in the estradiol cypionate-injected group. There were no significant differences between the cortisol-injected group and the peanut oil-injected control group or in the biological half life of cortisol between any groups.

There were no significant changes in the red colour of the flesh or in body weight during the experiment. However, the cortisol-injected group did show the largest weight loss.

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INTRODUCTION

SEXUAL MATURATION in the male Pacific salmon is accompanied by an increase in the volume of distribution and metabolic clearance rate of radioactivity after injection of a tracer dose of [4-^{14}C] cortisol (Donaldson and Fagerlund, 1968a, b). Salmon which were gonadectomized in July had volumes of distribution and metabolic clearance rates the following December which were similar to those observed in normal maturing salmon in August and early September. Thus gonadectomy in addition to preventing the death of these fish in November (McBride et al., 1963) also prevented the increase in volume of distribution and metabolic clearance rate (Donaldson and Fagerlund, 1968a, b).

When the actual metabolic clearance rate and secretion rate of cortisol was determined in one gonadectomized, two mature, and one spawned salmon, it was found that these parameters were lowest in the gonadectomized fish (Donaldson and Fagerlund, 1968b). To determine the causative factor or

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factors for the changes in cortisol dynamics observed during the maturation of the Pacific salmon the androgens 11-ketotestosterone and 17α-methyltestosterone and the corticosteroid cortisol were injected into groups of gonadectomized male salmon. The injection of androgen resulted in an increased volume of distribution, metabolic clearance rate, and resting secretion rate of cortisol in addition to causing the typical loss of flesh colour which occurs in maturing salmon (Fagerlund and Donaldson, 1969).

The purpose of this investigation has been to determine whether estrogens could also be causative factors in the changes in cortisol dynamics which accompany sexual maturation and whether they play a role in the mobilization of flesh colour.

MATERIALS AND METHODS

Fish — The fish used in this study were immature female sockeye salmon (*Oncorhynchus nerka*) which were captured during their spawning migration in July 1967, at the entrance to Great Central Lake, Vancouver Island, B.C. The fish were transported to the laboratory under mild anaesthesia and were given a prophylactic injection of antibiotic (Donaldson and Fagerlund, 1968a, b). Shortly after their arrival in the laboratory the fish were gonadectomized (McBride et al., 1963) and 3–4 weeks later the fish were induced to feed on a preparation of marine zooplankton (McBride, 1967). Throughout the experiment the fish were maintained on a natural photoperiod.

Hormone treatment — In January 1968, 38 salmon, which had a mean body weight of 1682 g, were divided into one group of 6 and four groups of 8 fish. The first group (six fish) served as zero time controls; group two received two intramuscular injections of 0.2 ml peanut oil twice weekly for 8 weeks; group three received 2.5 mg cortisol in 0.2 ml peanut oil twice weekly for 8 weeks; group four received 2.5 mg estradiol in 0.2 ml peanut oil twice weekly for 8 weeks; group five received 1.0 mg estradiol cypionate (Depo-Estradiol, The Upjohn Co., Don Mills, Ont.) in cotton seed oil once weekly for 8 weeks. During the injection period one peanut oil-injected fish and two cortisol-injected fish died.

Determination of cortisol dynamics — The volumes of distribution of the inner and outer compartments (V_1 and V_2), biological half life ($T\frac{1}{2}$), and metabolic clearance rate (MCR), of total dichloromethane extractable radioactivity and cortisol and the secretion rate of cortisol (S), were determined as described previously (Donaldson and Fagerlund, 1968b; Fagerlund and Donaldson, 1969). Plasma cortisol concentrations were determined by a fluorimetric technique developed for use in salmonid fish (Donaldson et al., 1968). The fluorimetric method has been tested to determine whether estradiol interferes with cortisol measurement. An amount equivalent to 500 μg estradiol/100 ml plasma gave a fluorescence reading after extraction equivalent to less than 1 μg cortisol/100 ml plasma. This fluorimetric technique should not be confused with less specific fluorimetric techniques, such as that used by Hill and Fromm (1968) in *Salmo gairdneri* which give higher apparent cortisol concentrations. The cortisol dynamics were determined for three fish in group one, five fish in groups two and three, four fish in group four, and five fish in group five. These and the remaining fish were used for a concurrent histological study by J. R. McBride.

Determination of body weight and flesh colour changes — All fish were weighed at the beginning and end of the injection period and weight changes were calculated as a percentage of the original body weight. The amount of red colour in the flesh was determined using a Gardner colour difference meter and expressed by the Hunter *a/b* value (Schmidt and Idler, 1958).

Statistical treatment of data — Experimental results for the various groups of fish have been expressed as mean ± standard deviation. Statistical comparisons between control and experimental groups have been made using Student's *t*-test.

RESULTS

CORTISOL DYNAMICS

Volume of distribution — The apparent volumes of the inner (V_1) and outer (V_2) pools, calculated for both total dichloromethane extractable $[C^{14}]$ and for cortisol $[C^{14}]$ have been calculated as a percentage of body weight and are shown in Fig. 1. V_1 for total dichloromethane extractable $[C^{14}]$ and cortisol $[C^{14}]$ were similar within each experimental group. V_1 for both total $[C^{14}]$ and cortisol $[C^{14}]$ was significantly larger in the estradiol- and estradiol cypionate-injected fish than in the peanut oil-injected control fish (Table 1). V_2 for cortisol $[C^{14}]$ was consistently higher than V_2 for total $[C^{14}]$ in each group. V^2 for total $[C^{14}]$ in the estradiol and estradiol cypionate groups and for cortisol $[C^{14}]$ in the estradiol group was significantly higher than V_2 for the control group (Table 1).

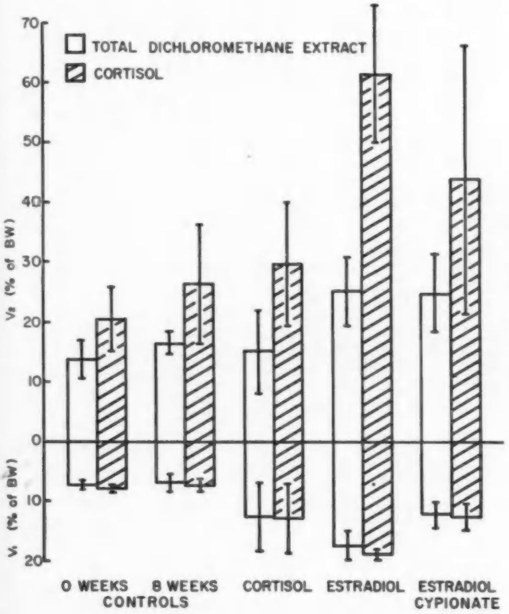

FIG. 1. Apparent volume of distribution (mean and standard deviation) of total dichloromethane extractable $[C^{14}]$ and cortisol $[C^{14}]$) in the inner pool, V_1, and the outer pool, V_2, after injection of $[4\text{-}C^{14}]$ cortisol into gonadectomized female *Oncorhynchus nerka* which had received injections of peanut oil or steroids for 8 weeks (BW = body weight.)

Biological half-life — The biological half life ($T_{\frac{1}{2}}$) for total dichloromethane extractable $[C^{14}]$ ranged from a mean of 108 min in the peanut oil-injected control group to 160 min in the cortisol-injected group. The $T_{\frac{1}{2}}$ for cortisol $[C^{14}]$ was shorter and ranged from 46 min in the peanut oil-injected control group to 68 min in the cortisol-injected group (Fig. 2). Although there were no significant differences in the mean values for cortisol $[C^{14}]$ $T_{\frac{1}{2}}$ between the

TABLE 1. Comparison of cortisol dynamics in gonadectomized female *Oncorhynchus nerka* receiving estrogens for 8 weeks with peanut oil-injected control fish.

	Control	No. of fish	Estradiol	No. of fish	P^a	Estradiol cypionate	No. of fish	P^a
Total dichloromethane								
Extractable [C^{14}] dynamics:								
$T_{\frac{1}{2}}$ *(min)*	107.7±19.5[b]	5	142.7±24.1	4	<0.05	121.0±32.8	5	NS[c]
V_1 *(% of body wt)*	6.9±1.6	5	17.2±2.3	4	<0.001	12.0±2.2	5	<0.005
V_2 *(% of body wt)*	16.5±2.0	5	25.2±5.8	4	<0.02	24.9±6.6	5	<0.05
MCR *(ml/kg body wt per hr)*	26.7±4.1	5	50.7±8.9	4	<0.001	42.0±4.3	5	<0.001
Cortisol dynamics:								
$T_{\frac{1}{2}}$ *(min)*	46.2±10.0	5	50.8±5.6	4	NS	55.4±13.8	5	NS
V_1 *(% of body wt)*	7.1±1.1	5	18.7±0.9	4	<0.001	12.4±2.3	5	<0.005
V_2 *(% of body wt)*	26.5±10.1	5	61.5±11.6	4	<0.005	44.0±22.5	5	NS
MCR *(ml/kg body wt per hr)*	66.0±12.9	5	153.8±15.8	4	<0.001	95.3±97.1	5	<0.02
Cortisol concentration *(μg/100 ml plasma)*:								
Resting	1.3±0.4	5	7.0±2.9	5	<0.005	3.1±5.0	5	NS
End of expt	36.4±15.2	5	14.9±2.7	5	<0.02	24.6±4.4	5	NS
Cortisol secretion rate *(μg/kg body wt per hr)*:								
Resting	0.9±0.5	5	10.0±4.4	4	<0.001	3.0±1.5	5	<0.02
End of expt	23.7±9.0	5	24.5±1.1	4	NS	23.7±7.1	5	NS

[a] Comparison with peanut oil-injected control.
[b] Standard deviation.
[c] Not statistically significant.

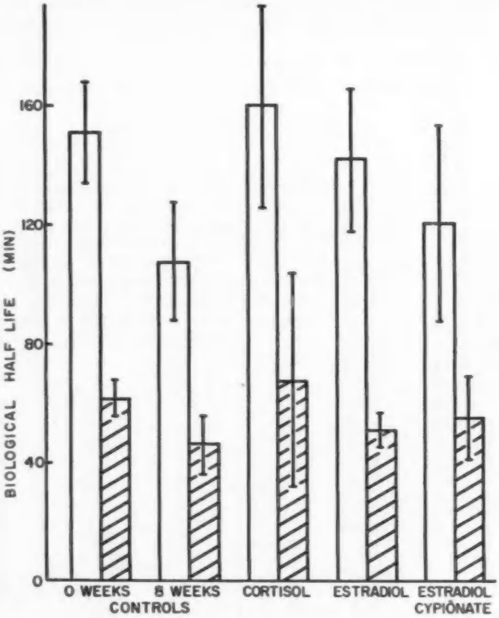

FIG. 2. Biological half life (mean and standard deviation) of total dichloromethane extractable [C^{14}] (plain bars) and cortisol [C^{14}] (hatched bars) after injection of [4-C^{14}] cortisol into gonadectomized female *Oncorhynchus nerka* which had received injections of peanut oil or steroids for 8 weeks.

various groups, the values for total [C^{14}] $T_{\frac{1}{2}}$ in the zero control ($P < 0.02$), cortisol-injected ($P < 0.02$), and estradiol-injected ($P < 0.05$) groups were significantly higher than in the peanut oil-injected control group (Fig. 2, Table 1).

Metabolic clearance rate — The metabolic clearance rate (MCR) of cortisol [C^{14}] was greater in each group than the MCR of total dichloromethane extractable [C^{14}] (Fig. 3). The total [C^{14}] MCR was significantly lower in the zero control fish ($P < 0.05$) and significantly higher in the estradiol- and estradiol cypionate-injected fish than in the peanut oil-injected control fish (Table 1). The cortisol [C^{14}] MCR was significantly higher in the estradiol- and estradiol cypionate-injected fish than in the peanut oil-injected control fish (Table 1).

Plasma cortisol concentration — The concentration of cortisol in the plasma was determined in the resting fish on the day before secretion rate determination, again at the beginning of the experiment after the fish had been anaesthetised, and finally at the end of the secretion rate determination when the fish had been anaesthetized for 6.5 hr. These blood samples were obtained from the caudal region (Donaldson and Fagerlund, 1968). The plasma cortisol concentration was significantly higher in the estradiol-injected fish than in the peanut oil-injected control fish when the fish were at rest and at the beginning of the experiment (Fig. 4, Table 1). The cortisol concentration

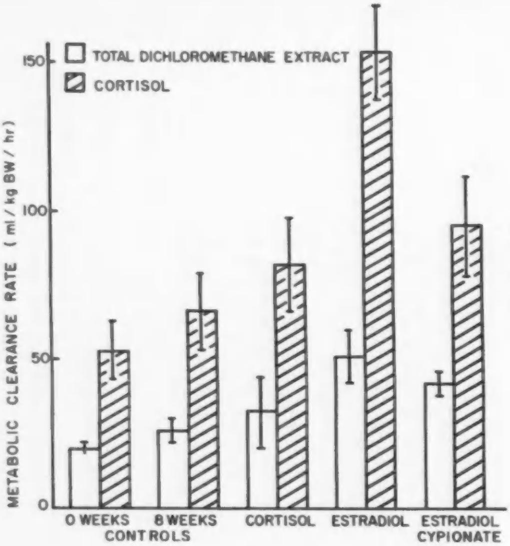

Fig. 3. Metabolic clearance rate (mean and standard deviation) of total dichloromethane extractable [C^{14}] and cortisol [C^{14}] after injection of [4-C^{14}] cortisol into gonadectomized female *Oncorhynchus nerka* which had received injections of peanut oil or steroids for 8 weeks. (BW = body weight.)

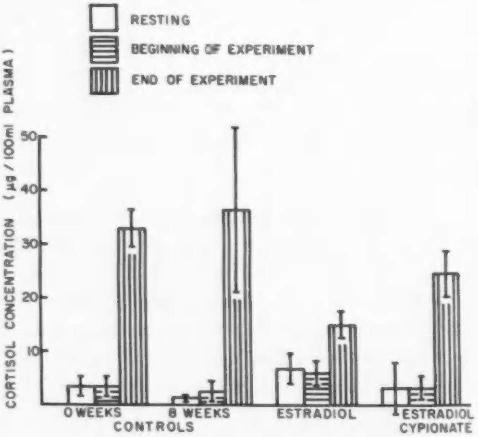

Fig. 4. Plasma cortisol concentration (mean and standard deviation) in gonadectomized female *Oncorhynchus nerka*, which had received injections of peanut oil or estrogens for 8 weeks measured while resting, after 30 min in anaesthetic solution (beginning of experiment) and 6 hr after injection of [4-C^{14}] cortisol (end of experiment).

in all groups was elevated at the end of the experiment; however, the estradiol-injected fish showed the smallest increase and had a mean plasma cortisol concentration which was significantly lower than the peanut oil-injected control fish (Table 1). The plasma cortisol concentrations for the cortisol-injected group are not reported as they reflect the biweekly cortisol injections rather than the functional status of the interrenal gland.

Cortisol secretion rate — The cortisol secretion rate was calculated both for fish in the resting state and at the end of the experiment (Fig. 5). The resting cortisol secretion rate was higher by a factor of 10 in the estradiol group compared with the peanut oil-injected control fish (Table 1). The cortisol secretion rates were higher in all groups at the end of the experiment and there were no significant differences between the peanut oil-injected control group and the other groups.

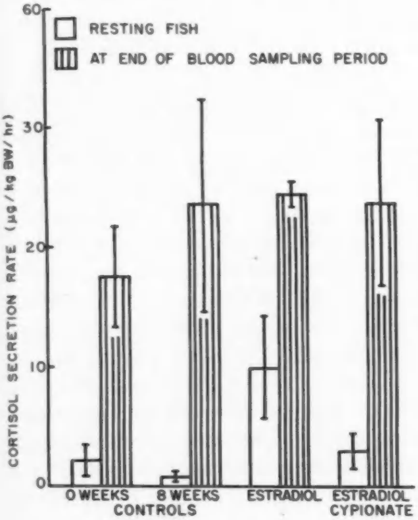

FIG. 5. Cortisol secretion rate (mean and standard deviation) in gonadectomized female *Oncorhynchus nerka*, which had received injections of peanut oil or estrogens for 8 weeks calculated in resting fish and 6 hr after injection of [4-C^{14}] cortisol (end of blood sampling period). (BW = body weight.)

BODY WEIGHT AND FLESH COLOUR

The mean body weight of the salmon in all groups was less at the end of the experiment than at the beginning (Fig. 6). The losses ranged from 2.1% in the estradiol-injected group to 7.7% in the cortisol-injected group. However, the differences between the control fish and the other groups were not significant.

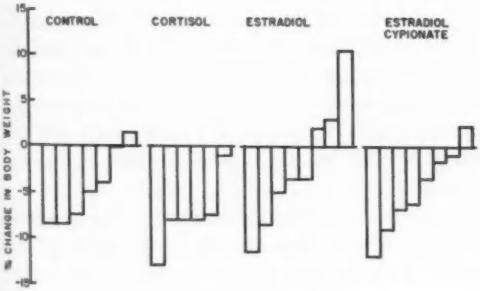

FIG. 6. Change in body weight of gonadectomized female *Oncorhynchus nerka* after injection of peanut oil or steroids for 8 weeks.

There were no significant differences in the flesh colour of the fish in the various groups when the colour was expressed as the Hunter *a/b* value (Fig. 7).

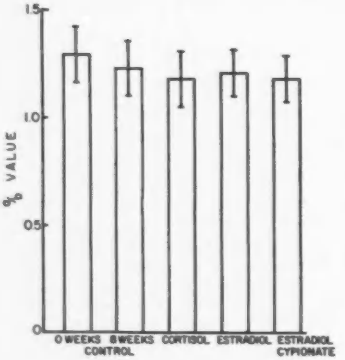

FIG. 7. Degree of redness of the flesh of gonadectomized female *Oncorhynchus nerka* which had received injections of peanut oil or steroids for 8 weeks expressed as the Hunter *a/b* value (mean and standard deviation).

DISCUSSION

The dynamics of cortisol in the peanut oil-injected control group of gonadectomized female sockeye salmon resemble quite closely the values reported earlier for peanut oil-injected gonadectomized male sockeye salmon (Fagerlund and Donaldson, 1969). The cortisol $T_{\frac{1}{2}}$ in the latter fish was 41.5 min compared with 46.2 min in the female salmon whereas V_1 and V_2 were 10.7 and 30.1% in the gonadectomized male fish compared with 7.2 and 26.5% in the gonadectomized female fish. The lower value for V_1 in the female fish is reflected in a lower cortisol MCR, 66.0 vs. 105.0 in the male fish. This lower cortisol MCR in the females may be a true sex difference or may be a result of the female salmon having a longer post-operative recovery period after gonadectomy than the male salmon.

Intraperitoneal implantation of pellets containing cortisol and cholesterol into starving *Salmo gairdneri* is reported to have resulted in marked histological changes in several tissues (Robertson et al., 1963), which resembled the changes that occur in spawning *Oncorhynchus tschawytscha* (Robertson et al., 1961). Injection of cortisol into fed gonadectomized female salmon (*O. nerka*) in the present experiment or into fed gonadectomized male salmon (Fagerlund and Donaldson, 1969) resulted in no significant changes in cortisol MCR such as occur in salmon injected with estrogen or androgen. Injection of cortisol into the female salmon did, however, increase the $T_{\frac{1}{2}}$ of total dichloromethane extractable [C^{14}] and this may reflect a small change in the rate of clearance of cortisone. A report on the histological changes occurring in these fish will be published later (McBride, in preparation).

The two groups of female salmon which received estradiol and estradiol cypionate both had higher V_1, V_2, MCR, and resting secretion rates than the peanut oil-injected control fish; however, the increases were greatest in the estradiol group probably as a result of the five-fold higher dose of estradiol compared with estradiol cypionate.

The significant increase in the resting plasma cortisol concentration after estradiol treatment indicates that interrenal activity in these fish is increased perhaps as a result of increased ACTH secretion by the pituitary gland which may indicate that the estradiol has reset the feedback control mechanism in the hypothalamus. Richard (1966) observed an increase in the basal plasma corticosterone concentration after implantation of estradiol into the arcuate nucleus, anterior pituitary, or lateral mamillary area of ovariectomized rats. Kitay (1963, 1968) noted an increase in pituitary ACTH content, adrenal weight, and adrenal venous blood corticosterone in female gonadectomized rats injected with polyestradiol phosphate. Thus, there are similarities between the response of the gonadectomized female salmon and rat to estrogen treatment. However, corticosterone production values in the rat determined by collection of adrenal venous blood are usually regarded as being maximal (Kitay, 1968). In the gonadectomized female salmon the resting cortisol production rate was higher in the estrogen-treated group than in the control group whereas the production rate, after the fish had undergone the stress of the experiment, was the same in both groups owing to the plasma cortisol concentration being lower in the estradiol-injected group.

The resting cortisol secretion rate in the fish which received estradiol was similar to that in methyltestosterone-injected gonadectomized male salmon (9.98 vs. 9.1 μg/kg body weight per hr) (Fagerlund and Donaldson, 1969) whereas the cortisol secretion rate calculated at the end of the experiment was lower in the estradiol-treated fish than in the methyl testosterone-injected salmon and similar to that in the 11-ketotestosterone-injected fish. Both 11-ketotestosterone (Idler et al., 1960) and estradiol (Botticelli and Hisaw, 1964) have been isolated from *O. nerka*. Thus the increase in the secretion rate of cortisol during the normal maturation of *O. nerka* may be a result of the combined effects of both androgen and estrogen.

Injection of ethynyl estradiol into humans resulted in an increase in the quantity of cortisol in the intravascular pool, but not in the extravascular pool (Bradley and Waterhouse, 1966). This effect in the human may be explained by the increased plasma transcortin concentration after estrogen treatment (Musa et al., 1965). Plasma binding of cortisol in female *Salmo salar* has been found to range from 3.68 to 6.72% when determined by gel filtration and between 4.95 and 5.68% when determined by double equilibrium dialysis (Freeman and Idler, 1966). There was no difference between ripe and spawned fish. More recent determinations using equilibrium dialysis show the cortisol in a single pool of immature *S. salar* blood to be 49.8% bound whereas that in a pool from mature fish was 34.5% bound (Idler and Freeman, 1968). A similar change was observed in male and female *O. nerka* during maturation and has been related to the decrease in total plasma protein concentration during the spawning migration (Idler and Freeman, 1968). On the other hand, injection of estradiol monobenzoate into maturing female *O.*

nerka resulted in an 18% increase in total plasma protein (Ho and Vanstone, 1961). Thus although injection of estradiol in the present experiment may have increased the concentration of plasma protein and thus the amount of weakly bound cortisol, the net effect was probably that of increasing the concentration of biologically active cortisol in the plasma.

The proposal which has been put forward that plasma cortisol concentrations are inversely proportional to biological half life (Freeman and Idler, 1966) does not hold true in *O. nerka* as estradiol treatment resulted in a significant increase in resting cortisol concentration, but no change in biological half life.

Recently estradiol injection has been shown to increase the resting plasma cortisol concentration in female freshwater yellow eels (*Anguilla rostrata*) and it has been proposed that, as these fish are in a state of inanition during their spawning migration, the estrogen-mediated increase in cortisol may stimulate gluconeogenesis and thus provide a source of energy during migration (Butler, 1968; Clarke, MS, 1968). This may also hold true in *Oncorhynchus* during its spawning migration. A study by Hane et al. (1966) showed an increase in the plasma concentration of 17-hydroxycorticosteroids during the spawning migration of *O. tschawytscha*, female salmon having the highest concentration. On the other hand Fagerlund (1967) did not find an increase in plasma cortisol concentration during the migration of *O. nerka*.

The lack of any change in the flesh colour of these fish when compared with the loss of flesh colour seen in androgen-injected salmon, indicates that it is the increasing concentration of androgen during the sexual maturation of these fish which is responsible for the loss of flesh colour in maturing *O. nerka*. Although there were no significant changes in body weight in the various groups, weight loss was greatest in the cortisol-treated group which is consistent with a gluconeogenic role for this hormone in the salmon. This effect of cortisol may have been more clear-cut if the fish had not been fed.

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Digestive Response of Rainbow Trout, *Salmo gairdneri*, to Pellet Diets[1]

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WINDELL, JOHN T., DAVID O. NORRIS, JAMES F. KITCHELL, AND JAMES S. NORRIS. Digestive response of rainbow trout, *Salmo gairdneri*, to pellet diets. J. Fish. Res. Bd. Canada 26: 1801–1812.

Quantitative data are presented for three laboratory experiments concerning rate of gastric evacuation of pellets (fed in gelatin capsules) and pellet components. Rainbow trout, *Salmo gairdneri*, acclimated to a constant water temperature of 15 C were killed 12 hr after consuming an experimental meal. Subtraction of amount of dry matter remaining at autopsy from dry matter consumed yielded amount of food digested or evacuated or both, from the stomach per unit time.

Meals of encapsulated pellet were evacuated from the stomach more rapidly (65.8% decrease in 12 hr) than encapsulated corn oil (42.6%), gelatin (50.8%), a combination of corn oil and gelatin (47.3%), saturated fat (28.8%), or methyl cellulose (50.3%).

Groups of fish consuming five capsules equal to approximately 0.65% of their body weight evacuated 35.9, 45.1, 64.2, 95.5, and 100% at intervals after killing from 6 to 36 hr, respectively. Similar groups consuming seven capsules equal to approximately 1.0% of their body weight evacuated 23.7, 57.9, 70.5, and 86.6% at intervals after killing from 4 to 20 hr, respectively.

Ten groups of trout consuming amounts of dry matter equal to 0.24, 0.40, 0.74, 1.11, 1.31, 1.19, 1.59, 1.56, 1.91, and 2.26% of their body weight evacuated 90.7, 81.3, 64.2, 57.9, 56.6, 52.5, 53.4, 51.3, 58.7, and 50.0% in 12 hr, respectively. Gastric motility remains relatively constant once food has entered the stomach. However, when larger meals are fed a greater quantity is evacuated per unit time. This could only be accomplished by changes in volume of gastric contents pumped per peristaltic stroke or number of strokes per unit time.

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INTRODUCTION

PRELIMINARY DATA on the length of time required by rainbow trout, *Salmo gairdneri*, to partially or completely evacuate the stomach of pelleted hatchery food have been reported by Windell and Norris (1969). Their data included 12 trout that were fed to satiation and may not be truly representative of the daily feeding practices in hatcheries. In recent years the feeding of fishes on all-concentrate diets, rich in protein, has become an established hatchery practice. On such diets, it is possible that at high levels of feeding, relatively large amounts of dry matter may escape gastric or intestinal digestion and

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therefore assimilation. Physiological demands upon the digestive system of a fish consuming dry pelleted feed or a meal of natural food organisms are obviously different. We are currently ignorant of the extent to which fishes can enzymatically handle artificially prepared foods. Providing artificial foods containing 90–95% digestible organic matter may pose special physiological problems when one considers that trout are adapted to natural foods containing approximately 5–20% digestible organic matter. In view of this lack of fundamental physiological information, attempts to determine the duration and fate of pellets in the alimentary canal at appropriate environmental temperatures are needed.

A variety of methods have been used to measure rate of gastric digestion. These include visual estimates of percentage decrease of food from the stomach (Armstrong and Blackett, 1966; Herting and Witt, 1968); measuring volume by water displacement (Hunt, 1960; Seaburg and Moyle, 1964); observing the disappearance of bony and other hard parts by X-ray (Molnár and Tölg, 1960, 1962; Molnár et al., 1967); and measuring at intervals the difference between weight of food intake and remains in the alimentary canal in terms of dried digestible organic matter (Windell, 1966, 1967, 1968; Kitchell and Windell, 1968; Windell and Norris, 1969).

This paper provides quantitative data about the rate of gastric evacuation of meals of encapsulated pelleted food and various pellet concentrates. We compared gastric emptying time for: (1) encapsulated $\frac{1}{8}$" pellets with encapsulated corn oil, gelatin, a combination of 25% corn oil and 75% gelatin, pure fat (lard), and cellulose; (2) a meal representing 0.65% consumed dry matter with one of 1.0% of the body weight; and (3) 10 groups of fish that consumed encapsulated pellet meals ranging from 0.24 to 2.26% of the body weight. The present work follows an earlier study on gastric digestion and evacuation in rainbow trout (Windell and Norris, 1969). Future papers will describe similar work with young trout in terms of: (1) food progression through the stomach and the effect of successive 4-hr meals upon an initial meal and subsequent meals; and (2) the gastric evacuation of oligochaetes compared with encapsulated pellets at water temperatures of 5, 10, 15, and 20 C.

METHODS AND MATERIALS

Rate of digestion was considered a function of gastric evacuation measured by utilizing a dry weight method. All computations were based on per cent dry weight of wet weight fed. Subtraction of dry matter remaining in the stomach at autopsy from dry matter consumed yielded the amount of food evacuated from the stomach per unit time. Food being evacuated from the stomach (i.e. mechanical removal) does not necessarily mean that it was digested (i.e. chemical change). There is evidence that some indigestible materials (e.g. chitin) may remain in the stomach for a longer period until ground into small pieces (Windell, 1966). Indigestible pellet components (i.e. fiber, ash, etc.) were ignored for the purposes of this study since they are already ground and may pose no problem with reference to gastric emptying.

The principal food substance used was Diamond "S" Trout Food $\frac{1}{8}$" pellets manufactured by Simpson and Company, Colorado Springs, Colorado. Guaranteed analysis of protein, fat, and fiber was 28, 6.0, and 5.0%, respectively.

Pellet disintegration in water was prevented by filling transparent #5 pure gelatin capsules with pellets and various other pellet ingredients. All experimental meals and dry weight analysis included the capsules. Large numbers of capsules were filled with one of the six previously named food substances and analyzed for wet, dry, and ash weights. Moisture content was determined by drying in an oven at 80 C until a constant dry weight was achieved. Ash content was determined by overnight ignition of a dry, previously weighed sample in porcelain crucibles in a muffle furnace at 650 C. Covers were placed over each crucible to prevent loss of material and ashing was continued until only a fine white residue remained.

Experimental apparatus used in this study was designed to maintain a constant water temperature of 15 C (±1.0). A 1 hp refrigeration unit with a 120-gal capacity storage tank was arranged in association with six troughs (12′ × 20″ × 12″). Water was circulated from the storage tank to each trough by siphoning and returned by float-controlled electric pumps. Each trough was divided by plexiglass partitions into six compartments that contained an individual fish in approximately 25 liters of aerated charcoal-filtered water. Rate of water flow through the entire system was 6–10 liters per min. Intensity and duration of fluorescent light were automatically regulated to simulate natural photoperiod during the month of each experiment (May, June, July 1968).

Hatchery-reared *Salmo gairdneri* were acclimated to the laboratory environment for at least 2 weeks and conditioned to voluntarily consume #5 gelatin capsules containing various food substances. Once all fish were thoroughly conditioned to voluntarily consume capsules, a 3-day fasting period was imposed to insure a complete absence of food remains from the stomach. After the fasting period each fish was allowed to consume an experimental meal of known composition weighed to the nearest 0.1 mg. All animals ate a full meal promptly. Groups of fish were killed and immediately autopsied at predetermined time intervals. Autopsy was identical to that described in previous studies with bluegills, *Lepomis macrochirus* (Windell, 1966, 1967, 1968), pumpkinseed sunfish, *Lepomis gibbosus* (Kitchell and Windell, 1968), and rainbow trout (Windell and Norris, 1969).

Care was exercised during killing to prevent regurgitation of stomach contents. The head was maintained in a vertical position during dissection. However, some results were disregarded because regurgitation occurred in spite of all precaution.

GASTRIC EVACUATION OF PELLETS AND PELLET COMPONENTS

The purpose of the following experiment was to compare the digestibility of pellet and individual meals of cellulose, corn oil, a combination of 25% corn oil and 75% gelatin, and pure fat (lard). An experimental meal included five #5 capsules filled with one of the six food substances. (Composition of capsules, pellets and pellet ingredients are given in Table 1.) All five capsules were consumed immediately after placement in the water compartment.

TABLE 1. Composition of whole pellet and pellet ingredients contained in pure gelatin capsules.

	Water (%)	Dry matter (%)	Ash (%)
Gelatin capsule, empty[a]	12.0	88.0	–
Corn oil	2.1	97.9	–
Pellet	6.6	93.4	8.9
Corn oil and gelatin[b]	7.7	92.3	0.8
Cellulose	2.2	97.8	–
Gelatin	7.0	93.0	1.1
Pure fat (lard)	0.0	100.0	–

[a] Mean wet weight per empty capsule was 26.6 mg.

[b] Percentage of each component used was 25% corn oil and 75% gelatin.

Experimental meals averaged 695 mg of wet weight and 608 mg of dry matter except the less dense meals of cellulose that averaged 285 mg and 279 mg, respectively (Table 2). Mean percentage of body weight consumed as dry weight was 0.72 for pellet, corn oil, corn oil and gelatin, pure fat, gelatin, and 0.30 for the less dense cellulose.

Per cent gastric evacuation at killing varied considerably for the six different kinds of experimental meals. The pellet-filled capsule meal displayed the most rapid exit from the stomach decreasing by 65% after 12 hr. Cellulose and gelatin meals decreased by 50.3 and 50.8%, respectively. Pure corn oil-filled capsules decreased more slowly (42.6%) than did a combination of corn oil and gelatin (47.3%). Gastric evacuation of pure fat was substantially retarded in comparison to the other experimental meals reaching only 28.8% decrease after 12 hr.

The significantly lower value for pure fat supports the contention by Windell (1967) that the high fat content of *Tenebrio molitor* larvae may have an inhibitory effect upon gastric emptying in some centrarchids. The inhibitory effect of fats, fatty acids, and many natural triglycerides on gastric emptying has been well documented (Roberts, 1931; Quigley and Meschan, 1941). Using cats, rats, and other mammals, unsaturated triglycerides were determined more retarding per gram than saturated (Tidwell and Cameron, 1942). Note that any substance in pure form was evacuated by the trout more slowly than the pellet mixture.

MEAL EVACUATION OF FIVE AND SEVEN CAPSULES

A pair of analogous experiments was conducted to determine rate of gastric evacuation of meals representing different percentages of body weight. Experimental conditions were identical for both experiments. In the first experiment 19 fish averaging 202 mm fork length and 90 g in weight consumed five capsules equal to approximately 0.65% of their body weight. Mean wet weight per meal was 640 mg and 596 mg of dry matter (Table 3). Groups of fish were killed and autopsied exactly 6, 8, 12, 24, and 36 hr after consumption of the experimental meal. Rate of gastric evacuation in per cent decrease averaged 35.9, 45.1, 64.2, 95.9, and 100 for the previously named intervals, respectively.

In the second experiment 18 fish averaging 203 mm fork length and weighing 84 g consumed seven capsules equal to approximately 1.0% of their body weight (Table 4). Mean wet weight per meal was 895.9 mg and 836.7 mg of dry matter. Groups of fish were killed and autopsied at 4, 12, 16, and 20 hr and mean rate of gastric evacuation was 23.7, 57.9, 70.5 and 86.6%, respectively.

Gastric evacuation progressed at approximately the same rate for the different size meals (Fig. 1). The average time required to digest 100% of the food was not determined precisely; however, it can be estimated from the data to be slightly greater than 24 hr. Like earlier studies the volume of

TABLE 2. Gastric evacuation of encapsulated pellets and pellet components. All fish were killed after 12 hr of digestion. Each group of fish voluntarily consumed meals of five (#5) gelatin capsules.

	No. of fish in expt.	Fish length (*mm*)	Fish wt (*g*)	Wet wt fed (*g*)	Dry matter fed (*g*)	% of food evacuated		Dry wt of body wt fed (%)
						Range	Mean	
Pellet	4	198	80	.5904	.5514	54.5–78.6	65.8	0.69
Corn oil	5	206	87	.6528	.6459	34.2–53.9	42.6	0.74
Gelatin	5	202	86	.5402	.5024	27.0–69.1	50.8	0.58
Cellulose	4	209	94	.2850	.2788	47.4–53.1	50.3	0.30
Pure fat (lard)	5	204	84	.7005	.7005	16.1–50.6	28.8	0.83
Corn oil (25) and gelatin (75)[a]	5	205	87	.6940	.6405	32.1–55.9	47.3	0.74

[a] Refers to percentage of each component used.

TABLE 3. Rate of gastric evacuation of ⅛" pellets contained in gelatin capsules at 15 C. All fish voluntarily consumed five capsules equal to approximately 0.65% of their body weight.

Hours after feeding	No. of fish	% of food evacuated		Dry wt of body wt fed (%)
		Range	Mean	
6	4	28.2–41.2	35.9	0.55
8	4	36.7–53.0	45.1	0.64
12	3	58.9–70.2	64.2	0.74
24	4	94.6–97.3	95.5	0.82
36	4	–	100.0	0.51

TABLE 4. Rate of gastric evacuation of ⅛" pellets contained in gelatin capsules at 15 C. All fish voluntarily consumed seven capsules equal to approximately 1.0% of their body weight.

Hours after feeding	No. of fish	% of food evacuated		Dry wt of body wt fed (%)
		Range	Mean	
4	5	19.3–27.2	23.7	0.89
12	3	53.9–61.1	57.9	1.11
16	5	60.5–79.1	70.5	1.11
20	5	80.2–93.5	86.6	0.91

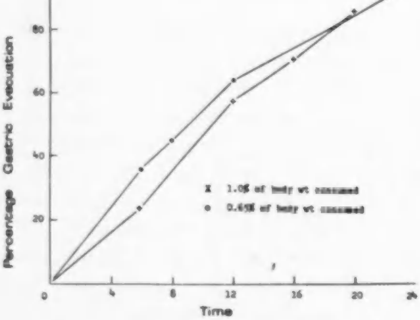

FIG. 1. Rate of gastric evacuation of encapsulated ⅛" pellet meals representing 0.65% and 1.0% of the fish body weight.

dry matter evacuated during a given interval increased in proportion to meal size (Hunt, 1960; Windell, 1966; Kitchell and Windell, 1968). When all other factors are equal (e.g. temperature) the stomach probably operates as a pump. The greater the volume received per unit time, the correspondingly greater the volume evacuated.

MEAL EVACUATION OF TWO TO SEVENTEEN CAPSULES

The following experiment was performed to determine rate of gastric evacuation of amounts of dry matter above and below the 1.0% level of fish body weight. Test meals were consumed by 10 groups of three or four fish each. Mean weight and fork length were 91 g and 203 mm, respectively (Table 5). Number of capsules consumed per fish ranged from 2 to 17 and represented dry matter extremes of 0.24 and 2.26% of the fish body weight. Size of meals consumed ranged between 234 mg and 2.108 g wet weight or 198 mg and 1.969 g of dry matter. All groups were autopsied 12 hr after consuming the test meal.

Groups consuming meals of dry matter of 0.24, 0.40, and 0.74% of their body weight evacuated from the stomach 90.7, 81.3, and 64.2%, respectively (Fig. 2 and Table 5). The remaining seven groups consumed meals of dry matter ranging from 1.11 to 2.26% of their body weight and gastric evacuation ranged between 50 and 60%, but no trend was apparent. The results from the latter seven groups suggest that gastric motility, once initiated remains relatively constant as long as food remains in the stomach. Since there was progressive increase in amount of dry matter consumed by each group and essentially the same percentage evacuated after 12 hr, it becomes clear that increased amounts of material were evacuated per unit time. This could only be accomplished by changes in the volume of gastric contents pumped per peristaltic stroke or the number of strokes per unit time. Hence, the phenomenon of regulation of gastric emptying in fishes (trout) is much like that of higher vertebrates and depends upon volume of gastric contents.

Hunt and Knox (1968) recognized that transfer of gastric contents to the intestine occurs intermittently in humans about three times per minute. They hypothesize that the frequency of the peristaltic cycle does not change, so that regulation of gastric emptying depends upon changes in the volume of gastric contents pumped per stroke. The data presented above substantiates this contention for rainbow trout fed encapsulated pellets.

There was a direct relationship between the amount of dry matter consumed and that evacuated from the stomach per hour (Fig. 3). For example, a 90-g fish that consumed 900 mg of dry matter or approximately 1.0% of its body weight evacuated approximately 45 mg/hr. Likewise, a similar fish that consumed 1800 mg (2% of its body weight) evacuated about 90 mg/hr. In both examples the original meal would have decreased by about 60% after 12 hr of digestion at 15 C.

Stomachs of fish consuming small amounts of pelleted food may empty at a rate near maximal. In this instance 90% of the smallest meal consumed (group I fish) was evacuated at the end of 12 hr. Small amounts of dry matter may become saturated with gastric juice more quickly forming a dilute mixture thereby facilitating gastric removal. As the size of the meal increased in proportion to the fish body weight, the volume emptied decreased until leveling off between 50–60%. However, fish feeding to satiation (e.g. group X) may suffer from the opposite effect of those consuming small amounts. Gastric evacuation in terms of milligrams per hour appeared to be retarded when the dry matter content of a meal exceeded 1.91% of the fish body weight. Stomachs probably empty at a near minimal rate as a result of being satiated.

TABLE 5. Rate of gastric evacuation of increased amounts of ⅛″ pellets contained in gelatin capsules fed to rainbow trout, *Salmo gairdneri*, at 15 C. All fish were killed 12 hr after feeding.

Group	No. of capsules consumed	No. of fish in in expt.	Mean fish wt (*g*)	Mean fish length (*mm*)	Wet wt fed (*g*)	Dry wt fed (*g*)	% of food evacuated		Mean dry wt of body wt fed (%)
							Range	Mean	
I	2	4	84	203	0.2338	0.1976	87.5–93.5	90.7	0.24
II	3	4	94	203	0.4022	0.3756	65.4–94.3	81.3	0.40
III	5	3	83	197	0.6565	0.6132	58.9–70.2	64.2	0.74
IV	7	3	78	192	0.9270	0.8658	53.9–61.1	57.9	1.11
V	9	4	83	195	1.1651	1.0882	48.2–65.2	56.6	1.31
VI	11	4	108	216	1.3752	1.2844	53.5–58.4	52.5	1.19
VII	12	3	88	202	1.4853	1.4006	52.5–56.3	55.4	1.59
VIII	14	3	107	215	1.7903	1.6721	44.8–57.9	51.3	1.56
IX	16	3	97	210	1.9840	1.8531	53.8–63.6	58.7	1.91
X	17	1	87	199	2.1080	1.9688	50.0–	50.0	2.26

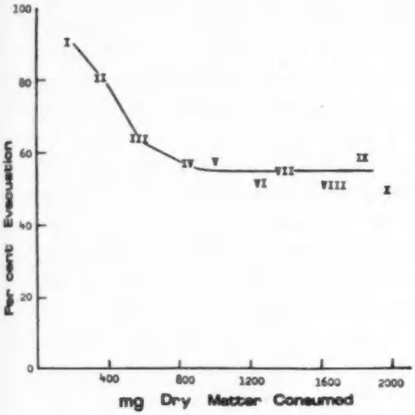

FIG. 2. Rate of gastric evacuation of encapsulated pellets by 10 groups of trout consuming amounts ranging from 0.24 to 2.26% of their body weight. All fish were held at constant water temperatures of 15 C and killed 12 hr after feeding. Roman numerals represent the 10 groups.

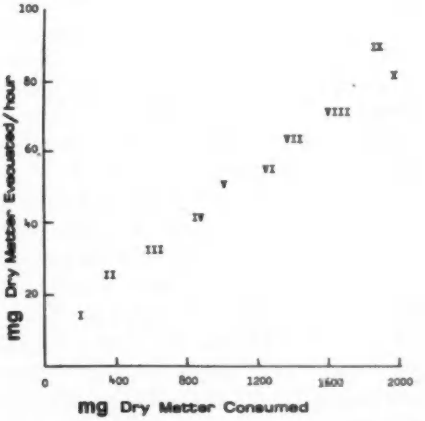

FIG. 3. Relationship of milligrams dry matter consumed to milligrams dry matter evacuated per hour. Roman numerals represent the 10 groups.

This same phenomenon was observed in earlier experiments (Windell and Norris, 1969). Increased demands for additional gastric juice saturation of the pellet may not have been met physiologically and resulted in prolonging evacuation.

VARIATION

Individual variation in the amount of dry matter evacuated after 12 hr varied for each of the experiments (Table 2–5). Greatest variation occurred among the groups of fish consuming encapsulated pellet components, i.e., corn oil. Less variation occurred among individual fish when meals of encapsulated pellets were consumed (Tables 3–5) and was the same magnitude as that for *L. macrochirus* (Windell, 1966) and *L. gibbosus* (Kitchell and Windell, 1968). Variation was very pronounced, 27.0–69.1% decrease, in the group consuming pure gelatin capsules filled with pure gelatin. Likewise, considerable individual variation occurred between fish consuming pure fat-filled capsules. It may be that these substances are not only evacuated from the stomach more slowly but can result in greater individual digestive variation than encapsulated pelleted foods.

Hunt (1960) found that individual variation in the time required to completely evacuate the stomach of a meal of fish became very pronounced as the terminal period approached. In the present experiments no attempt was made to assess variation during the terminal period. However, when 0.65 and 1.0% of the body weight was consumed, fish killed at 20 and 24 hr, respectively, did not show extreme variation (Tables 3 and 4).

DISCUSSION

A fish stomach may hold its contents until a certain degree of liquefaction and digestion is reached, and then the contents are moved into the intestine. In man, food begins to leave the stomach 2 or 3 min after it has been swallowed (Wilson et al., 1929). This was not the case in the present experiments. The gelatin capsules containing the $\frac{1}{8}''$ pellets are insoluble in water. They do soften and become pliable but require enzymatic hydrolysis before the pellet material can be digested. Individual empty capsules placed in agitated .2 M HCl/KCl buffer solution (*p*H 2.1) required about 35 min for complete disintegration. Capsules fed to white grunt, ***Haemulon plumieri***, were almost completely intact after 2 hr but were mostly dissolved after 4 hr (Smith, 1967).

Viscosity of the stomach contents may not be a factor affecting emptying time. In humans, viscosity of the gastric contents makes no difference to the rate or pattern of emptying (Hunt, 1954). Mastication obviously solves part of the viscosity problem. Fish consuming pelleted food appear not to have a viscosity problem since the pre-ground ingredients are easily liquified in water. This suggests that within certain limits gastric evacuation proceeds independently of gastric digestion. The importance of this suggestion leads logically to a study of assimilation. As the stomach is emptied, the ingredients making up the pellets may be an important factor to consider with relation to motility and secretion. Evidence for an inhibitory action on gastric motility and secretion after the ingestion of fats is conclusive for *S. gairdneri* and many other animals. Introduction of glucose solutions above a certain concentration into the intestine of dogs also inhibited the gastric secretory response (Day and Komarov, 1939). Studies designed to elucidate physiological effects of concentration levels of consumed fats, oils, glucose, etc. in pelleted food may be meaningful to produce a truly superior artificial food.

The volume of gastric juice secreted in relation to meals of pelleted food may be an important factor in efficiency of food conversion by fishes. There is no reason to believe that digestion and gastric evacuation are directly comparable in a physiological sense for meals of aquatic insect larvae and encapsulated pellets. A major difference lies in the amount of dry matter consumed with each type of meal. A trout ingesting a full meal of pellets containing 93% dry matter consumes more than six-fold the amount contained in a meal of oligochaetes representing only 16% dry matter. Still to be ascertained is whether the amounts of gastric juice produced to digest a full meal of natural food is sufficient to digest a full meal of pellet. Before this and related questions can be answered the quantitative analysis of hydrochloric acid and pepsinogen secretion under a variety of meal conditions and volumes must be investigated.

The possibility of enzyme saturation does exist. Fish stomachs containing volumes of dry matter in excess of 1.0% of the body weight are moving the excess through the gut at a relatively constant rate. This excess may be only partially acted upon enzymatically. If this is true, the question of efficiency of assimilation becomes important. Visual inspection of gastric and intestinal contents of numerous well-fed hatchery-reared trout suggests this possibility should be investigated since gut contents in the lower portion of the canal resemble pure pellet material. There may be a need to investigate the potential benefits of added inactiviated enzyme to commercial pellet diets. The increased enzyme could facilitate more complete digestion thereby enhancing assimilation and growth.

Windell (1966) found that gastric evacuation of natural food organisms in *Lepomis macrochirus* was characterized by three stages: (1) a brief initial lag; (2) a surge of gastric activity and movement when the bulk of the meal was emptied from the stomach; and (3) a period of several hours when the remaining small amount of digesta was removed. This latter period suggested gastric motility and evacuation were not efficient when a small amount of food was in the stomach. Results from these and subsequent experiments reveal similar information and can be related to anatomical studies (Oppel, 1896) for at least partial explanation. A dense collagenous layer is found in the gut wall of a number of vertebrates including salmonid fish. This layer greatly reduces the extensibility of the gut wall (Burnstock, 1959) and could well affect the ability of stretch receptors to initiate peristalsis. As long as sufficient food is present in the stomach to stimulate the stretch receptors, peristalsis occurs at a fixed rate. When a small amount of food is present, the gut wall is not stretched and peristalsis is either not initiated or weakly initiated. This may be an adaptative mechanism for processing small amounts of food more efficiently and thereby allowing time for greater assimilation.

The natural stimulus initiating peristalsis in rainbow trout is still open to question and has not been ascertained in the gut of lower vertebrates (Campbell and Burnstock, 1968). Peristalsis did occur in the gut of brown trout when it was distended (Burnstock, 1958). Although little information is available (Barrington, 1957), peristaltic initiation undoubtedly is related to a combination of distention of the gut wall with chemical or tactile stimulation of the mucosa. Texture of artificial foods may be a factor to consider in the initiation of gastric juice secretion and volumes secreted.

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Effects of Temperature and Body Weight on Endogenous Nitrogen Excretion in the Bluegill Sunfish (*Lepomis macrochirus*)[1,2]

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The effects of temperature and body weight on endogenous nitrogen excretion (ENE) rate were examined for fish acclimated at 7.2, 15.6, 23.9, and 29.4–32.2 C. Nitrogen excretion rates were very high at the highest temperature and decreased with a decrease in acclimated temperature from 29.4–32.2 C to 15.6 C. Nitrogen excretion rates were equal at 15.6 and 7.2 C. From these data, an estimate of maintenance protein for a population of bluegill sunfish (*Lepomis macrochirus*) was calculated on a seasonal and yearly basis.

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INTRODUCTION

THE PURPOSE of this investigation was to determine the protein maintenance level for bluegill sunfish (*Lepomis macrochirus*) at 7.2, 15.6, 23.9, and 29.4–32.2 C. From these data, an estimate of maintenance protein for a population of bluegill sunfish was calculated on a seasonal and yearly basis.

The maintenance level was determined by measuring endogenous nitrogen excretion (ENE). ENE is the amount of nitrogen excreted when a fish is fed a nonprotein diet — in these experiments, glucose. ENE is directly related to the amount of body protein utilized for energy, and this protein must be replaced before growth can occur.

The estimate of the maintenance level for a population of bluegill sunfish was facilitated by the occurrence of equal nitrogen excretion rates at 15.6 C and 7.2 C. This physiological adjustment is called capacity adaptation (Precht, 1958). Physiological adjustments to very high or low temperatures are resistance adaptations (Precht, 1958). No high temperature resistance adaptation was found in bluegills.

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METHODS

BODY WEIGHT AND TEMPERATURE

Nitrogen excretion rates were measured for 20 fish at 7.2 C, 14 fish at 15.6 C, 40 fish at 23.9 C, and 15 fish at 29.4–32.2 C. Acclimation of the larger bluegills to 32.2 C was difficult. During preliminary experiments a few large bluegills died at this temperature. No deaths occurred among the smaller fish. Nitrogen excretion rates for bluegills weighing 50 g or more were measured at 29.4 C.

In August 1965, 12 fish were used at 23.9 C and 8 fish at 32.2 C. During the summer of 1966, the remaining data on nitrogen excretion rates were determined for bluegills at all four temperatures. Some measurements at 23.9 C were made during the spawning season; however, the fish used were not in reproductive condition. Measurements at 29.4 C were made prior to spawning, and all other measurements were made after spawning.

The bluegills were caught by seining at Bear Lake in Noble County and Tippecanoe Lake in Kosciusco County in Indiana. Only fish from Bear Lake were used in experiments at 29.4–32.2 C. For experiments at 23.9 C, four fish were obtained from Tippecanoe Lake, and the other fish from Bear Lake. Fish from Tippecanoe Lake were used at 15.6 C and 7.2 C.

To acclimate fish at 29.4–32.2 C, 15.6 C, and 7.2 C, the temperature was raised or lowered 1.1 C from the initial starting temperature of 23.9 C each day until the desired temperature was reached. During temperature acclimation, fish were fed mealworms, *Tenebrio moliter*. After reaching the experimental temperature, a 3-day starvation period was initiated to reduce the protein reserve. Protein reserve consists of plasma proteins and proteins stored in body organs which can be used to maintain the balance between catabolism and anabolism (Brody, 1945, p. 353).

At the end of the starvation period, each fish was anesthetized with 0.5% urethane, blotted with a wet cloth to remove excess moisture, weighed to the nearest 0.1 g, and placed in an aerated aquarium. Fish also received a daily injection of 0.5 g/ml glucose solution into the stomach. For these glucose injections, a glass tube was attached to a syringe and was gently moved through the mouth cavity and esophagus into the stomach. The tube was fire-polished at one end and had an outside diameter of 4 mm. The amount of glucose injected was calculated from an example provided by Gerking (1955b). Glucose can be absorbed by the digestive tract and utilized for energy by fish (Phillips et al., 1948).

There was only one fish per aquarium. Each aquarium contained a known quantity of water and was covered with a glass plate to minimize evaporation. Aerated well water was used, and numerous analyses indicated that the water contained no measurable amount of organic nitrogen or ammonia. Fish between 10 and 30 g were placed in 6 liters of water; larger fish were placed in 8–11 liters of water. The aquaria were kept in constant temperature rooms with either a fluorescent light of 40 w or a 60-w incandescent bulb for illumination. The photoperiod was automatically controlled to provide 12 hr of light and 12 hr of darkness.

Nitrogen excretion was measured either 3 or 5 days after the fish were placed in their separate aquaria. Duplicate 25-ml samples were pipetted from each aquarium and digested following the procedure of Bruel et al. (1947). The organic nitrogen and ammonia were measured quantitatively by the microkjeldahl method and were expressed as milligrams nitrogen excreted per day.

SOURCES OF ERROR

The 3-day starvation period may not have reduced the protein reserve substantially. Morgulis (1918) found no stabilization of nitrogen excretion after 4 weeks of starvation on brook trout, *Salvelinus fontinalis*, and concluded that protein reserve had not been depleted. Data showing a stabilization of nitrogen excretion rate for bluegills 7 days after the beginning of starvation are given in Table 1 (Savitz, MS, 1967).

Some glucose may have been regurgitated, thus decreasing the amount of glucose available for the metabolism of the fish. Gerking (1955b) found no regurgitation of glucose using this experimental method, but on a few occasions during the present experiments regurgitation was observed.

Table 1. The nitrogen excretion rates for four different starvation periods.

Starvation period (*days*)	No. of fish	SE of mean nitrogen excretion rate	Mean rates with 95% confidence limits (*mg N/day*)
7	6	±0.65	4.89±1.67
14	10	±0.57	6.35±1.29
21	9	±0.70	5.63±1.61
28	10	±0.94	6.66±2.13

Gerking (1955b) calculated the amount of glucose which was equivalent in calories to the metabolic rate of bluegills at 25.9 C. Since the metabolic rate of poikilotherms changes with temperature, the caloric value of glucose injected may have been below the metabolic rate of bluegills at 29.4–32.2 C. An additional quantity of glucose, 0.1–0.2 ml, was injected into eight fish at this temperature. No difference between the fish injected with the additional quantity and the other fish was apparent in the results.

Nitrogen excretion is largely in the form of ammonia, but it is not lost by aeration under the conditions of the experiment (Gerking, 1955b). Another possible source of error is the organic nitrogen originating from mucus secretion; the amount and rate of secretion are unknown. Job (1955) observed that anesthetizing brook trout, *Salvelinus fontinalis*, with urethane caused excessive mucus secretion. The inclusion of mucus nitrogen biases the results toward a higher metabolic rate at all temperatures. The periodic handling of the fish may also have caused an increase in nitrogen excretion. Variability in the results may be due to individual differences in nutritional and thermal history and activity during the experiments.

Bluegills may not have been fully acclimated at 7.2, 15.6, and 29.4–32.2 C. To acclimate fish at 7.2 C, 15 days would be required to reach the temperature with an additional 3 days acclimation during which fish were starved. Consequently, there was a total of 18 days for a change of 16.7 C. Roberts (1966) feels reasonably sure that the 90% level of acclimation is reached in 1 week by sunfish above 15 C and within 2 weeks at lower temperatures.

RESULTS

The relationship between metabolism and body weight can be expressed as

$$Y = aW^b$$

or

$$\log Y = \log a + b \log W$$

where Y is metabolic rate, W is body weight, b is the weight exponent, and a is the y-axis intercept (Brody, 1945, p. 365). The same relationship exists between metabolic rate, in terms of nitrogen excretion, and body weight (Brody, 1945, p. 374).

The weight exponents were 0.99 at 29.4–32.2 C, 0.98 at 23.9 C, 0.93[4] at 15.6 C, and 0.96 at 7.2 C (Table 2). The weight exponent values are nearly

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[4] This value differs from that found in Savitz's (MS, 1967) dissertation. Data from six fish in the fall of 1965 were eliminated from the present paper since all other measurements were taken during the summers. Metabolism during the fall for bluegills is lower than in the summer (Wohlschlag and Juliano, 1959).

TABLE 2. Linear equations calculated from the log of body weight and the log of nitrogen excretion rate for fish acclimated at four different temperatures.

Temp (*C*)	No. of fish	Linear equation	SE
29.4–32.2	15	$\log y = -0.52550+0.99063 \log x$	±0.075
23.9	40	$\log y = -0.89079+0.97656 \log x$	±0.101
15.6	14	$\log y = -1.17675+0.93138 \log x$	±0.300
7.2	20	$\log y = -1.17483+0.95979 \log x$	±0.160

the same at all temperatures. Paloheimo and Dickie (1966), after an extensive review of the literature, concluded that the weight exponent is relatively constant in metabolic studies of fish and does not change when examined at different constant temperatures.

The nitrogen excretion rates with their 95% confidence limits were calculated for the average 100-g and 10-g bluegill at each temperature (Table 3). The ENE rate for the average 100-g bluegill is 28.56 mg N/day (23.18–35.20) at 29.4–32.2 C, 11.54 mg N/day (9.46–14.08) at 23.9 C, 4.85 mg N/day (2.26–10.43) at 15.6 C, and 5.55 mg N/day (3.98–7.76) at 7.2 C.

TABLE 3. Mean nitrogen excretion rates and 95% confidence limits for the mean rates for 100- and 10-g bluegills at four different temperatures.

	100 g		10 g	
Temp (*C*)	Mean rate (*mg N/day*)	95% confidence limits	Mean rate (*mg N/day*)	95% confidence limits
29.4–32.2	28.56	23.18–35.20	2.92	2.60–3.27
23.9	11.54	9.46–14.08	1.21	0.94–1.58
15.6	4.85	2.26–10.43	0.57	0.22–1.46
7.2	5.55	3.98–7.76	0.61	0.40–0.90

The nitrogen excretion rates are exceptionally high at 29.4–32.2 C and significantly different when compared with rates at other temperatures (Table 3). The average nitrogen excretion rates decrease with a decrease in acclimated temperature from 29.3–32.2 C to 15.6 C but are equal at temperatures of 15.6 C and 7.2 C (Table 3). The physiological adjustment by poikilotherms which results in similar metabolic rates at two different temperatures is called capacity adaptation (Precht, 1958).

The 95% confidence limits of nitrogen excretion rates of fish at 15.6 C slightly overlap those at 23.9 C (Table 3). This result is partially due to the small number of fish above 60 g used at 15.6 C (Fig. 1). Only 14 fish of which 3 were above 60 g were used for the calculation of the equation at 15.6 C. This small number has resulted in larger confidence limits. The actual data for the larger fish show lower nitrogen excretion rates at 15.6 C than at 23.9 C (Fig. 1).

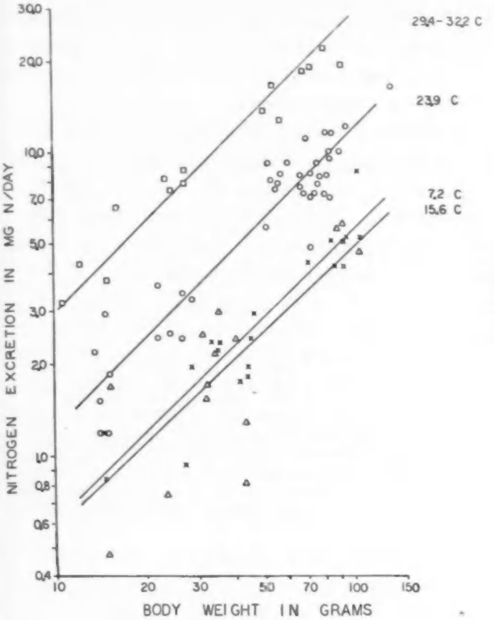

FIG. 1. The relationship between ENE rate and body weight at four different temperatures. □ = 29.4–32.2 C; ○ = 23.9 C; △ = 15.6 C; × = 7.2 C.

DISCUSSION

Relation of ENE Rates to Other Metabolic Studies

Paloheimo and Dickie (1966) found that a weight exponent of 0.8 describes the relationship between metabolic rate and body weight for most fishes. Wohlschlag and Juliano (1959), measuring oxygen consumption of bluegills at different seasons, found a weight exponent of 0.85. The weight exponents at the four temperatures used in these experiments ranged from 0.93 to 0.99. These values are similar to those obtained by oxygen consumption experiments. Weight exponents of ENE and of basal metabolism are also similar in homeotherms (Terroine and Sorg-Matter, 1928; Smuts, 1938).

Gerking (1955b) measured ENE rates of bluegills and found a weight exponent of 0.54. There is a large amount of variability between individual fish in Gerking's experiment and this experiment. This variability may possibly be due to the nutritional and thermal history of the fish and individual differences in activity during the experiments.

ENE — A Conservative Measure of Protein Maintenance Level

The protein maintenance level determined with glucose-fed fish should be lower than with protein-fed fish. With a protein diet, the maintenance level is influenced by the quantity and quality of the proteins as well as the protein–calorie ratio (Morrison and Rao, 1967). Mitchell (1929) found that 0.030 g N/kg body weight was needed for maintenance level in homeotherms as determined by ENE measurements. But 0.060 g N/kg body weight was needed for maintenance level with most protein diets.

A conservative estimate of maintenance level is also obtained with ENE measurements when compared with feeding experiments (Fig. 2). Gerking (1955a) found that the extrapolated maintenance level for the average 27.9-g bluegill is 7.18 mg N/day (4.33–9.08). If one uses the equation for ENE at 23.9 C, the maintenance level is 3.32 mg N/day (2.79–4.18). A calculation of maintenance levels was made from Gerking's earlier results (1952) on a longear sunfish, *Lepomis megalotis*, and a green sunfish, *Lepomis cyanellus*, 103.3 and 48.5 g respectively. The longear sunfish had a maintenance level of 34.34 mg N/day, the green sunfish 23.86 mg N/day. Again, if one uses the equation for ENE at 23.9 C, these fish would have maintenance levels of 11.91 and 5.56 mg N/day. The difference between maintenance levels for fish fed a diet containing protein and those receiving glucose is clearly evident. This difference may also be influenced by species differences and activity of fish.

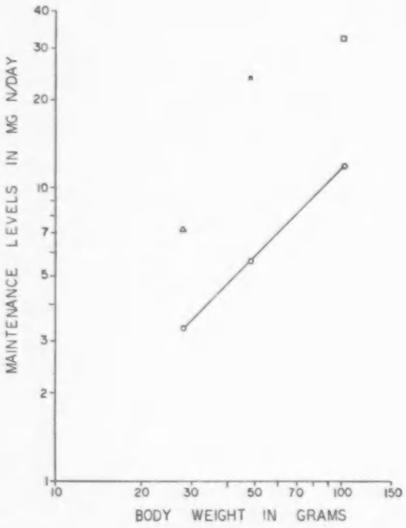

FIG. 2. Maintenance nitrogen levels calculated from feeding experiments and ENE experiments. ○ = bluegills (ENE experiments); △ = bluegills (feeding experiments); × = green sunfish (feeding experiment); ☐ = longear sunfish (feeding experiment).

TEMPERATURE ADAPTATION

A poikilotherm demonstrating resistance adaptation would have sharp decreases in metabolic rate at very low acclimated temperatures and high acclimated temperatures (Precht, 1958). Northern Indiana bluegills do not exhibit high temperature resistance adaptation at 29.4–32.2 C. Nitrogen excretion rate was not measured below 7.2 C and only speculation on low temperature resistance adaptation is possible. Low temperature resistance adaptation has been demonstrated for the green sunfish (Roberts, 1967).

The similar nitrogen excretion rates at 7.2 and 15.6 C provide an example of capacity adaptation. There are several hypotheses concerning the manner in which capacity adaptation occurs. One explanation is an increase in spontaneous activity. Wohlschlag and Juliano (1959) found oxygen consumption of bluegills was greater in summer and winter than in spring and fall. They observed an increase in spontaneous activity among several fish in the winter. If spontaneous activity is relatively important for thermal compensation in fish, protein must provide a substantial source of energy for activity.

Das and Prosser (1967) found a net accumulation of total protein in fish tissues during cold acclimation. In some tissues, such as gills and liver, reduced protein breakdown may cause the increase in protein, whereas increases in muscle protein may be due to an increase in the rate of protein synthesis (Das and Prosser, 1967). If most of the nitrogen excreted comes from the muscles, there is an increase in both synthesis and breakdown of muscle protein.

ESTIMATION OF MAINTENANCE LEVEL FOR A POPULATION OF BLUEGILLS

An estimate of the maintenance level was determined for a population of bluegills on a yearly and seasonal basis. Population estimates and average weights are available for age-groups II, III-slow growing, III-fast growing, IV, and V in Wyland Lake in 1956 (Gerking, 1962). The age-group distribution of the population which was available to the sampling method was as follows: age-group II, 210 fish (average weight 20 g); age-group III slow, 3670 fish (27 g); age-group III fast, 1560 fish (46 g); age-group IV, 760 fish (61 g); age-group V, 60 fish (66 g).

During the summer, the fish could live from the surface to 3 or 4 m. Adequate oxygen was not available below these depths. The temperatures from the lake surface to 3 or 4 m, taken at 1-m intervals, were available from June through August (Gerking, 1962). The average 3-month summer temperature was calculated at 23.4 C.

Temperatures for the other months at Wyland Lake were not available but should correspond generally to other lakes in the vicinity where numerous measurements have been made. In Sylvan Lake, Noble County, the temperature dropped from 23 to 18 C during September, from 18 to 13 C during October, and from 13 to 7 C during November. From December through March the temperature ranged from 4 to 2 C. The temperature rose rapidly in April to 12 C and was 19 C by the end of May (Wetzel, 1966).

The maintenance level from September through May is calculated from the equation at 7.2 C. There is no significant difference in nitrogen excretion rates at 15.6 and 7.2 C. If fish had lower rates at 4 C, there would be an overestimate of the winter maintenance level. The maintenance level for the summer months is calculated from the equation at 23.9 C.

The nitrogen excretion rate is calculated for the average weight of each age-group at 7.2 and 23.9 C and then summed (Table 4). At 7.2 C the total population excretes 13.04 g N/day and at 23.9 C, 26.60 g N/day. The latter rate is multiplied by 92 days (June through August) to obtain the total amount excreted in this period, 2847 g N. Nitrogen excretion rate is 73.04 g N/day during the other 9 months or 273 days. There would be 3560 g N excreted during this period. To determine the amount of protein needed for maintenance level, 2447 g N were added to 3560 g N and multiplied by the conversion factor, 6.25. During the year, 37.54 kg of body protein is replaced. This population used 15.29 kg of protein in the summer and 2.43 kg of protein for any 30-day period from September through May.

TABLE 4. ENE rates for each age-group and for a population of bluegills at 23.9 and 7.2 C.

Age-group	No. of fish	Avg wt (g)	ENE (*g/day*)	
			23.9 C	7.2 C
II	210	20	0.504	0.250
III slow	3670	27	11.781	5.799
III fast	1560	49	8.440	4.134
IV	760	61	5.441	2.630
V	60	66	0.461	0.224
			26.597	13.037

Gerking (1962) calculated replacement protein for this Wyland Lake population for a 5-month period using a linear equation for ENE at 25.9 C. His estimate was 34.4 kg protein from May through September. Using the linear equation at 7.2 C for 2 of these months and the linear equation at 23.9 C for 3 months, one obtains a value of 20.26 kg protein. The major difference in the two sets of results is that there was no data available on ENE at temperatures below 25.9 C when Gerking (1962) calculated the maintenance protein for the population. If the linear equation at 23.9 C is used for the 5-month period, the protein maintenance level would be 25.4 kg protein, 9 kg or 26.2% lower than Gerking's calculations.

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Stimulation of Sodium Transport Across the Isolated Toad Bladder by 1α-Hydroxycorticosterone from an Elasmobranch

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The mineralocorticoid activity of 1α-hydroxycorticosterone, an interrenal steroid in elasmobranch fish, was determined by the in vitro toad bladder bioassay. It stimulated the transport of sodium across the toad bladder at concentrations of 10^{-6}, 10^{-7}, and 10^{-8} M. The elasmobranch steroid had approximately 80% of the activity of *d*-aldosterone; it was slightly less active than aldosterone and corticosterone at the lower concentrations. Protein binding alone does not seem to explain the relative potencies of the two steroids. The concentrations of 1α-hydroxycorticosterone required to stimulate sodium transport across the toad bladder are comparable with those reported in the peripheral blood plasma of *Raja radiata* and *R. ocellata*. A seasonal variation in the number of positive responses was observed.

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INTRODUCTION

IN TWO ELASMOBRANCH SPECIES 1α-hydroxycorticosterone is the principal plasma corticoid and the principal steroid produced from endogenous precursors by in vitro interrenal gland incubations (Idler and Truscott, 1966a, b; Idler et al., 1967b). Furthermore, Truscott and Idler (1968) have recently reported the occurrence of a corticoid 1α-hydroxylase enzyme in the interrenals of a total of 11 elasmobranch species. The function of 1α-hydroxycorticosterone has not been established but the steroid is a potent mineralocorticoid, having 70% of the sodium-retaining activity of deoxycorticosterone in mouse and rat bioassays (Idler et al., 1967a). The present study is a further investigation into the mineralocorticoid properties of 1α-hydroxycorticosterone using an in vitro toad bladder method devised by Ussing and Zerahn (1951) and modified by Sharp and Leaf (1964).

MATERIALS AND METHODS

Large female toads, *Bufo marinus*, were obtained from National Reagents, Main Street, Bridgeport, Conn., USA. The animals were not fed but were held in darkened wooden boxes in a mixture of moist earth and sphagnum. At least 48 hr prior to use animals were half immersed in 1 liter of 0.11 M NaCl in individual plastic buckets.

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The apparatus used to measure sodium transport was similar to the double chamber modification (Sharp and Leaf, 1964) of the method of Ussing and Zerahn (1951). This technique measures the current produced by a hemi-bladder mounted across a "Lucite" chamber. Under short-circuit conditions the current produced by the hemi-bladder is a measure of sodium transport. The chambers were filled with a modified sodium Ringer's solution at *p*H 7.6, containing NaCl 113 mM, KCl 3.5 mM, $NaHCO_3$ 2.8 mM, $CaCl_2$ 0.89 mM, Na_2HPO_4 2 mM, *d*-glucose 2 mM. In some experiments, additional energy sources (glucose and pyruvate) were added to the medium before addition of steroids to the bladder, in an unsuccessful attempt to enhance the relative bladder response.

d-Aldosterone and corticosterone were obtained from Ikapharm, Israel. 1α-Hydroxycorticosterone was produced as described by Idler and Truscott (1966a). All steroids were made up in a small volume of methanol and added to the fluid bathing the serosal surface of the bladder. The final concentration of methanol never exceeded 0.1%. Amphenone and metopirone were very kindly supplied by Ciba Company, Ltd., Dorval, Que. Both compounds were added to the saline in which toads were soaked, at a concentration of 0.2 g/liter.

Bladder response was measured by dividing the maximum short-circuit current generated after steroid addition by the minimum short-circuit current generated during the latent period immediately after the addition of steroid.

All statistical methods used in evaluating the data were taken from Steel and Torrie (1960).

RESULTS

Figure 1 shows response patterns at steroid concentrations of 10^{-6} 10^{-7}, and 10^{-8} M in three hemi-bladders from different animals. In each case one-quarter was treated with 1α-hydroxycorticosterone and the other quarter with *d*-aldosterone. The response pattern of quarter bladders treated with 1α-hydroxycorticosterone closely resembles that of *d*-aldosterone-treated quarter bladders. After the addition of steroid there is a delay varying from 20–120 min before the increase in sodium transport (short-circuit current). Sodium transport reaches a maximum in approximately 3–5 hr and usually remains elevated for several further hours. The behaviour of the bladder in response to 1α-hydroxycorticosterone is similar to that found by Sharp and Leaf (1964) for *d*-aldosterone.

Using the toad bladder assay the mineralocorticoid activity of 1α-hydroxycorticosterone was compared with *d*-aldosterone and with the parent compound, corticosterone. In both instances 1α-hydroxycorticosterone was compared with the other two steroids at a concentration of 10^{-6}, 10^{-7}, and 10^{-8} M. Repeated attempts (eight) to induce a bladder response at steroid concentrations lower than 10^{-8} M were unsuccessful.

The relative activities of 1α-hydroxycorticosterone, *d*-aldosterone, and corticosterone are shown in Tables 1 and 2. In both tables two criteria were used when deciding whether or not to include the results from a particular hemi-bladder. Firstly, only those hemi-bladders in which both quarters responded to steroid were included (Table 1A, 2A). Secondly, all hemi-bladders in which either quarter responded to steroid were included (Table 1B, 2B). A response was taken as positive when there was an increase in short-circuit current at sometime after addition of steroid, and measured as a ratio as described previously. In Tables 1B, 2B, when only one side of the bladder responded positively, the negative response of the other side was recorded

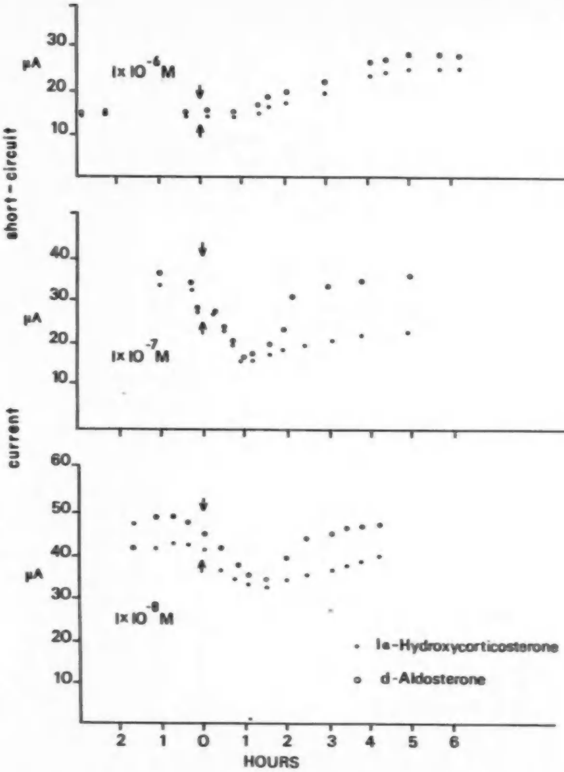

FIG. 1. Responses of three hemi-bladders to *d*-aldosterone and 1α-hydroxycorticosterone at concentrations of 10^{-6}, 10^{-7}, and 10^{-8} M. Arrows represent the time of steroid addition. Responses were measured in microamps (μA).

as a ratio of one. In both tables the mean difference and "single-tailed *t*-test" were used to compare the steroids. In Table 1A, 1α-hydroxycorticosterone stimulated sodium transport significantly less than *d*-aldosterone at a concentration of 10^{-7} M only ($P<0.005$). At a concentration of 10^{-6} and 10^{-8} M there was no significant difference in the activity of the two steroids. However, in Table 1B, 1α-hydroxycorticosterone stimulated sodium transport significantly less than *d*-aldosterone at 10^{-7} and 10^{-8} M ($P < 0.005$ at 10^{-7} M, and $P < 0.025$ at 10^{-8} M).

In Table 2, the mineralocorticoid activity of 1α-hydroxycorticosterone and corticosterone (B) are compared. In Table 2A, corticosterone was significantly more active than 1α-hydroxycorticosterone at all concentrations ($P < 0.05$ at 10^{-6}, $P < 0.025$ at 10^{-7}, $P < 0.005$ at 10^{-8} M). In Table 2B, corticosterone was more active than 1α-hydroxycorticosterone at a concentration of 10^{-8} M only ($P < 0.005$).

TABLE 1. Hemi-bladder response to *d*-aldosterone (*d*-ald.) and 1α-hydroxycorticosterone (1α) at 10^{-6}, 10^{-7}, and 10^{-8} M. Responses were measured in microamps as described in the text.[a]

	10^{-6} M			10^{-7} M			10^{-8} M		
	d-ald.	1α	Mean difference	*d*-ald.	1α	Mean difference	*d*-ald.	1α	Mean difference
(A) When both quarter bladders responded positively	3.0±0.3	3.0±0.3	0.09±0.3	3.0±0.6	2.0±0.3	0.9±0.3	1.5±0.1	1.4±0.1	0.08±0.08
	$n^b = 18$	$n = 18$ N.S.[b]		$n = 16$	$n = 16$ $P < 0.005$[c]		$n = 10$	$n = 10$ N.S.	
(B) When either quarter bladder responded positively	2.9±0.3	2.8±0.3	0.1±0.2	3.0±0.6	1.8±0.3	1.2±0.4	1.6±0.1	1.2±0.1	0.3±0.1
	$n = 19$	$n = 19$ N.S.		$n = 20$	$n = 20$ $P < 0.005$[c]		$n = 17$	$n = 17$ $P < 0.025$[c]	

[a] Each figure is given as mean ± standard error.

[b] n = number of quarter bladders tested; N.S. = no significant difference.

[c] Difference significant at the $P < 0.05$ level.

TABLE 2. Hemi-bladder response to corticosterone (B) and 1α-hydroxycorticosterone (1α) at 10^{-6}, 10^{-7}, and 10^{-8} M. Responses were measured in microamps as described in the text.[a]

	10^{-6} M			10^{-7} M			10^{-8} M		
	B	1α	Mean difference	B	1α	Mean difference	B	1α	Mean difference
(A) When both quarter bladders responded positively	2.4±0.4	2.0±0.5	0.4±0.2	1.9±0.3	1.6±0.3	0.3±0.1	1.7±0.1	1.5±0.1	0.2±0.06
	$n^b = 14$	$n = 14$ $P < 0.05^c$		$n = 8$	$n = 8$ $P < 0.025^c$		$n = 13$	$n = 13$ $P < 0.005^c$	
(B) When either quarter bladder responded positively	2.0±0.2	2.1±0.1	−0.1±0.1	1.6±0.1	1.5±0.1	0.07±0.2	1.5±0.1	1.3±0.1	0.2±0.05
	$n = 22$	$n = 22$ N.S.[b]		$n = 19$	$n = 19$ N.S.		$n = 18$	$n = 18$ $P < 0.005^c$	

[a] Each figure is given as mean ± standard error.

[b] n = number of quarter bladders tested; N.S. = no significant difference.

[c] Difference significant at the $P < 0.05$ level.

Note that Tables 1 and 2 each contain groups of different hemi-bladders treated with the same concentration of 1α-hydroxycorticosterone. It can be seen that the response of different hemi-bladders to the same concentration of 1α-hydroxycorticosterone can differ considerably. Comparisons were therefore restricted to groups of pairs of adjacent quarter bladders from the same hemi-bladders. Bearing this in mind we have refrained from making comparisons between the activity of corticosterone and *d*-aldosterone and between different concentrations of the same steroid in Tables 1 and 2.

There seems to be a definite dose–response relationship for 1α-hydroxycorticosterone and corticosterone, the response decreasing with the concentration. In Table 3, the regression lines and coefficients of correlation are shown when the response was plotted against the negative logarithm of the dose.

TABLE 3. Statistical data for regression lines of log dose and response for 1α-hydroxycorticosterone, *d*-aldosterone, and corticosterone.

	N No. of quarter bladders	Regression equation	Coefficient of correlation (r)	Significance of correlation
1α-Hydroxycorticosterone	46[c]	$Y = 7.97 - 0.83x$	−0.49	$P < 0.001$[a]
	35[d]	$Y = 3.95 - 0.31x$	−0.43	$P < 0.02$[a]
d-Aldosterone	54	$Y = 6.74 - 0.6x$	−0.25	N.S.[b]
Corticosterone	35	$Y = 4.78 - 0.39x$	−0.53	$P < 0.01$[a]

[a] Difference significant at the $p < 0.05$ level.
[b] N.S. = no significant difference.
[c] Quarter bladders compared with aldosterone.
[d] Quarter bladders compared with corticosterone.

A good linear relationship between log dose and response was observed for 1α-hydroxycorticosterone, the correlation being significant in both instances ($P < 0.001$ and $P < 0.02$). Corticosterone also showed a good linear relationship, the correlation being significant ($P < 0.01$). *d*-Aldosterone, however, did not have a significant correlation coefficient.

Differences in the response of different hemi-bladders to the same steroid are further seen in Table 4 in which hemi-bladder responses in 4 different months are compared. When treated with 1α-hydroxycorticosterone at 10^{-6} M the response of the June group of hemi-bladders is significantly greater than the August hemi-bladders ($P < 0.01$); also the activity of May hemi-bladders exposed to 10^{-8} *d*-aldosterone is significantly greater than the corresponding July hemi-bladders ($P < 0.05$).

In addition to the difference in the activity of different groups of hemi-bladders, there is evidence for a refractory period of the year during which the number of hemi-bladders responding to steroid are much reduced. In Table 5, the number of positive responses over a period of time are shown for

Table 4. Hemi-bladder responses to the same concentration of steroid during May to August 1967. Responses were measured in microamps as described in the text.[a]

Month	Steroid concentration	
	10^{-6} M 1α	10^{-8} M *d*-ald.
May	2.1±0.36 *n*[b] = 3	1.8±0.24 *n* = 8
June	3.0±0.32 *n* = 15	1.7±0.20 *n* = 6
July	2.0±0.14 *n* = 2	1.3±0.08 *n* = 10
Aug.	2.0±0.15 *n* = 24	–

[a] Each figure is given as the mean ± standard error.
[b] *n* = number of hemi-bladders.

each steroid. The mean concentration of the steroid added to the test bladder is given for each month, so that any decrease in response due to addition of low concentration of steroids throughout any month can be observed. During October 1966 and 1967, there were few positive responses with each steroid used (ca. 13% for 1967). There were no positive responses from the 15 bladders tested with *d*-aldosterone in October 1967. There was also a decrease in positive responses in September 1967, for the two steroids tested.

Table 6 shows the effect of pre-treatment of toads with adrenal inhibitors during 3 different months. Amphenone, an inhibitor of steroidogenesis and metopirone (CIBA), a steroid 11β-hydroxylase inhibitor, were added to the saline in which the toads were immersed at least 48 hr prior to excision of the bladder. Controls consisted of bladders from animals soaked in saline without metopirone and amphenone. Unfortunately, during October when the bladder response was lowest, metopirone was not used. In 8 out of 10 groups more of the metopirone-treated bladders responded to steroid. Overall, 50% of the treated bladders responded compared with 36% of the controls.

DISCUSSION

Since the final concentration of steroid in the assay system is known precisely and since the steroid is not subject to clearance and other phenomena, the in vitro bladder method appears to offer certain advantages over in vivo assay systems. It is possible to equate the concentration of steroid required to stimulate sodium transport in the assay with the concentrations normally found in the parent species. It is of particular interest that the concentrations of 1α-hydroxycorticosterone required to stimulate sodium transport, i.e. 10^{-8} – 10^{-7} M (0.36–3.6 μg/100 ml), are very similar to the concentrations found by Idler and Truscott (1966a) in the plasma of the skates *Raja radiata* and

TABLE 5. Seasonal variation in number of positive responses from bladders treated with *d*-aldosterone, 1α-hydroxycorticosterone, and corticosterone.

	1966		1967										1968			
	Oct.	Nov.	Jan.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.	May
							d - Aldosterone									
No. of bladders tested	15	5	11	3	27	41	18	–	–	15	5	–	–	–	6	30
Mean concentration[a] of steroid	10^{-7}	$10^{-7.4}$	$10^{-6.9}$	$10^{-6.6}$	$10^{-7.4}$	$10^{-6.7}$	$10^{-7.2}$	–	–	10^{-7}	$10^{-6.4}$	–	–	–	10^{-7}	$10^{-7.5}$
Positive responses[b] %	33	80	80	100	82	85	83	–	–	0	100	–	–	–	100	70
							1 α - Hydroxycorticosterone									
No. of bladders tested	–	–	7	1	19	34	26	59	25	11	11	16	22	19	–	–
Mean concentration of steroid	–	–	$10^{-5.7}$	10^{-5}	$10^{-7.1}$	$10^{-6.6}$	$10^{-7.4}$	$10^{-6.8}$	$10^{-7.9}$	$10^{-7.4}$	$10^{-6.5}$	$10^{-7.3}$	$10^{-7.2}$	$10^{-7.0}$	–	–
Positive responses %	–	–	86	100	63	76	88	61	36	9	64	50	64	79	–	–
							Corticosterone									
No. of bladders tested	–	–	–	–	–	–	5	56	21	8	–	–	–	–	–	–
Mean concentration of steroid	–	–	–	–	–	–	10^{-7}	$10^{-6.3}$	10^{-8}	$10^{-7.5}$	–	–	–	–	–	–
Positive responses %	–	–	–	–	–	–	100	54	43	13	–	–	–	–	–	–

TABLE 6. The effect of metopirone and amphenone on the proportion of hemi-bladders responding to steroid during June, August, and September 1967.

	June								Aug.								Sept.			
	1α 10^{-7} M		1α 10^{-8} M		*d*-ald. 10^{-7} M		*d*-ald. 10^{-8} M		1α 10^{-7} M		1α 10^{-8} M		B 10^{-7}		B 10^{-8}		1α 10 M^{-8}		B 10^{-8} M	
	+M[a]	−M	+M	−M	+M	−M	+M	−M	+M	−M	+M	−M	+M	−M	+M	−M	+M	−M	+M	−M
No. of hemi-bladders used	5	5	1	4	7	5	1	4	7	3	7	3	7	3	7	3	16[b]	3	16[b]	3
No. of positive responses	5	1	0	1	6	4	1	2	3	2	2	0	5	1	3	1	5[c]	0	7[d]	1
% of hemi-bladders giving positive responses	100	20	0	25	86	80	100	50	43	67	29	0	71	33	43	33	31	0	44	33

[a] +M = treated with metopirone and amphenone; −M = control hemi-bladders not treated with metopirone or amphenone.
[b] Includes 10 hemi-bladders from toads treated with amphenone only.
[c] Includes 3 hemi-bladders from toads treated with amphenone only.
[d] Includes 5 hemi-bladders from toads treated with amphenone only.

R. ocellata (0.5–2.0 μg/100 ml). Furthermore, 1α-hydroxycorticosterone is the principal corticoid so far isolated from the plasma and is the prominent product of in vitro interrenal gland incubation (Idler and Truscott, 1966a, b; Idler et al., 1967b). The possibility that 1α-hydroxycorticosterone functions as a mineralocorticoid in the parent species has been discussed by Idler et al. (1967a), but conclusive evidence must await a test system for evaluating the steroid in elasmobranchs (Idler and Szeplaki, 1968). Sodium and potassium gradients across the body wall of marine elasmobranchs are such that animals will tend to accumulate both cations. If then 1α-hydroxycorticosterone does function in the skate as a mineralocorticoid one might expect it to play a sodium or salt-excreting role in contrast to the sodium-retaining action of aldosterone and deoxycorticosteron ein mammals. Indeed, Mayer et al. (1967) have established that cortisol is a potent sodium-excreting factor in the seawater-adapted eel.

It is interesting to observe the differences in significance obtained in Tables 1 and 2 according to which criteria is used for including a particular bladder response. Because of these differences it was decided to include both criteria in the table. When aldosterone and 1α-hydroxycorticosterone are compared in Table 1B, when either quarter bladder responded positively, a significant difference was obtained at concentrations of 10^{-7} and 10^{-8} M. However, when the criteria was that both quarter bladders responded positively, a significant difference was obtained at 10^{-7} M only. This difference is due to the greater number of negative responses to 1α-hydroxycorticosterone, which increased the mean difference and therefore the significance. In Table 2A when 1α-hydroxycorticosterone and corticosterone are compared, there were significant differences in activity at all concentrations when the criteria demanded that both quarter bladders respond to steroid. However, when all bladders in which one quarter responded positively were counted, the only significant difference in activity was at 10^{-8} M. In this instance there were several negative values for corticosterone at 10^{-7} and 10^{-8} M which decreased the mean difference and therefore the significance. It is also interesting to note that the greatest differences in activity between the steroids are at the lower concentrations of 10^{-7} and 10^{-8} M. At a concentration of 10^{-6} M, little difference could be detected, which is understandable as at this concentration all the steroids had reached their maximum response.

While there is a significant difference between the activity of 1α-hydroxycorticosterone and the other two steroids (corticosterone and *d*-aldosterone) at 10^{-7} and 10^{-8} M, *all three steroids have the same general level of activity in the toad bladder assay.* Porter and Edelman (1964) using a similar assay system could not distinguish any significant difference in the activities of aldosterone, deoxycorticosterone, and cortisol at 7×10^{-7} M. These findings are in marked contrast to the relative sodium-retaining activities of these steroids found by in vivo mammalian assay methods. Idler et al. (1967a) have shown that 1α-hydroxycorticosterone has approximately 70% of the sodium-retaining activity of deoxycorticosterone when assessed by an in vivo rodent assay and deoxycorticosterone has between 1 and 10% of the sodium-retaining activity of aldosterone (Desaulles et al., 1953; Mattox et al., 1953). On this basis alone one might have expected 1α-hydroxycorticosterone to have approximately 0.7–7% of the activity of aldosterone. Instead, in the toad assay 1α-hydroxycorticosterone has approximately 80% of aldosterone activity. The large differences in the relative activities of 1α-hydroxycorticosterone and aldosterone calculated from different assay methods cannot be explained by the protein binding of steroids in in vivo methods. If it is assumed that only free steroid is biologically active, then using the values of Idler et al. (1967a) for total protein binding, free 1α-hydroxycorticosterone should have only 12% of the activity of free deoxycorticosterone in vitro. The difference between 1α-hydroxycorticosterone and aldosterone activities would then be increased by a further factor of 10 and the in vitro assay does not confirm this. It is also interesting to note the relatively high mineralocorticoid activity of corticosterone in this assay compared with in vivo mammalian assay methods where it has been found to have only a minimal effect on salt retention (Tait et al., 1952; Desaulles et al., 1955). Factors other than protein binding, such as metabolism and excretion, no doubt contribute to activity differences in the in vivo methods. The relatively high potency of 1α-hydroxycorticosterone in vivo suggests that it gets to the site of action in vivo with less loss than does corticosterone. Species differences in steroid potency may also be a factor (Gaunt and Chart, 1962).

A variable and sometimes large proportion of toad bladders failed to respond to exogenous steroid. There was also a particularly marked reduction in the number of bladder responses during October 1967, and also during September–October 1966. G. W. G. Sharp (personal communication) has also noticed a similar reduction from September–December. Although we do not understand the reasons for variability in response of individual animals and apparent seasonal variation, certain points are perhaps worthy of note. Aldosterone is present in normal plasma of *Bufo marinus* at levels of 0.68–1.17 μg/100 ml (Crabbé, 1963). High endogenous aldosterone levels might saturate the bladder leaving it refractory to any exogenous steroid. Although the animal husbandry was specifically aimed at maintaining low plasma aldosterone levels, a further attempt to reduce plasma aldosterone was made by means of "chemical adrenalectomy," using amphenone and metopirone as stated previously, but there was only a small improvement. However, it has yet to be established that refractory toads have a high plasma aldosterone level. High plasma aldosterone should stimulate sodium transport so that freshly excised bladders from refractory animals should show a higher short-circuit current than responsive bladders. No such difference in short-circuit current was observed in freshly excised bladders. This suggests that toads with refractory bladders did not have high endogenous steroid levels. In this event amphenone and metopirone treatment would not be expected to produce more responsive bladders. The possibility that reduced Na-K dependent ATPase activity might produce refractory bladders should be further investigated since Kennedy and Naylor (1965) have reported a marked seasonal variation in enzyme activity in toad cardiac muscle.

In our hands steroid concentrations less than 10^{-8} M did not result in short-circuit currents in excess of that prevailing when the steroid was added. Sharp and Leaf (1964) found that bladders responded to *d*-aldosterone at 3.6×10^{-9} M and regarded the lower limit of effective concentration to be between 3.3×10^{-10} and 1×10^{-10} M. The place of origin, supplier, maintenance, and preparation of the animals were the same in both studies and do not seem to explain the difference in sensitivity of bladders.

Now it would seem worthwhile to investigate a possible role for 1α-hydroxycorticosterone in the transport of sodium via the rectal gland of elasmobranchs. A unique role for 1α-hydroxycorticosterone with reference to urea seems less likely because the interrenal of the ratfish (*Hydrolagus colliei*), which also utilizes urea to regulate osmotic pressure, produces cortisol (Idler et al., 1969).

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Immunohistochemical Localization of ACTH and Prolactin in the Pituitary Gland of Adult Migratory Sockeye Salmon (*Oncorhynchus nerka*)

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McKeown, B. A., and A. P. van Overbeeke. 1969. Immunohistochemical localization of ACTH and prolactin in the pituitary gland of adult migratory sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 26: 1837–1846.

Antibodies to porcine adrenocorticotrophic hormone (ACTH), Synacthen (synthetic corticotrophin, Ciba), and ovine prolactin were prepared in rabbits and the antisera were tested for specificity against several pituitary hormones. The gamma-globulin fractions of the antisera were conjugated with fluorescein isothiocyanate and the labelled antibodies were "purified" by column chromatography.

Fresh-frozen sections of pituitary glands of adult migratory sockeye salmon were incubated with the antibody solutions and examined with a fluorescence microscope. The resulting antigen–antibody complex could be localized by re-photographing the same or alternate sections after fixation and staining. Anti-ACTH and anti-Synacthen appeared to be bound specifically by the epsilon cells, whereas anti-prolactin reacted with the eta cells of the rostral pars distalis. Pituitary glands collected at various stations along the migratory route, including one seawater sample, showed the same reactivity. Other glands were prepared for histological examination. Microspectrophotometric analysis of cell types showed that the granule density of the ACTH cells increased gradually during the later part of migration. In the prolactin cells, no change in granulation could be detected during entrance into the river or subsequent spawning migration.

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INTRODUCTION

The application of immunohistochemical techniques in localizing pituitary hormones was first reported by Marshall (1951), who was able to identify the adrenocorticotrophic hormone (ACTH) cells in the hog pituitary gland. Since then, several investigators successfully used this method in localizing a number of hormones in the pituitary glands of a variety of mammals. Thus corticotrophs were also identified by: Leznoff et al. (1962), Pearse and van Noorden (1963), McGarry et al. (1964), Hachmeister and Kracht (1965), Kracht et al. (1965, 1966), and Brozman (1967). Direct evidence regarding the cellular site of prolactin production was presented by Emmart et al. (1963, 1965) and Shiino and Rennels (1966). Moreover, Emmart and co-workers showed that antibodies to ovine prolactin are capable of binding a prolactinlike fraction prepared from pituitary glands of two teleost fishes: carp and pollack. They demonstrated, furthermore, that the same antibodies, conjugated to fluorescein isothiocyanate, are specifically bound to the cells of the rostral pars distalis in the teleost *Fundulus heteroclitus* (Emmart et al., 1966; Emmart

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and Mossakowski, 1967). To our knowledge, relative studies concerning the localization of ACTH cells in teleosts have not been published.

We have used the fluorescent antibody technique as a means to identify hormone-producing cells in the pituitary gland of sockeye salmon. This report concerns the localization of ACTH and prolactin in relation to the cytophysiology of the gland during anadromous migration and spawning.

MATERIALS AND METHODS

HORMONE PREPARATIONS

Porcine ACTH (Sigma, 140 IU/mg), Synacthen ($\beta^{1\text{-}24}$-corticotrophin, Ciba), and ovine prolactin (Sigma, 17 IU/mg) were used as antigens.

IMMUNIZATION PROCEDURE

Three groups of four New Zealand white rabbits were injected with ACTH, Synacthen, or prolactin respectively, and two rabbits were used as uninjected controls. Antigens were dissolved in 0.15 M NaCl in concentrations of 0.83 mg/ml (ACTH), 0.31 mg/ml (Synacthen), and 2.50 mg/ml (prolactin). Prior to injection each solution was mixed with an equal volume of Freund's complete adjuvant (Hyland Lab.). Injections were given intramuscularly or subcutaneously once every week. Three days after the fourth injection the rabbits were bled from the ear marginal vein and the antibody titer estimated. When the titer was sufficiently high (usually 7 weeks after the first injection), larger samples of approximately 30 ml were taken. Bleeding was performed once a week between injections. Ten days after the last injection most rabbits were chloroformed and exsanguinated from the jugular veins. Some animals were kept for 7 months after injections were stopped. The antibody titer in these rabbits gradually decreased, but one single injection was able to restore the titer to a high level within 10 days. The schedule of immunization and bleeding is shown in Table 1.

TABLE 1. Schedule of immunization of rabbits with three pituitary hormones.

		Volume of injection (*ml*)		
Day	Route of injection	Adjuvant	Hormone solution[a]	Bleedings
1	Intramuscular	0.5	0.5	
8	"	0.75	0.75	
15	"	1.0	1.0	
22	"	1.0	1.0	
25				Marginal vein
29	"	1.0	1.0	
32				"
36	"	1.0	1.0	
39				"
43	Subcutaneous	1.0	1.0	
46				"
53				Marginal vein or exsanguinated
133	Subcutaneous	0	1.0	
140				Exsanguinated

[a] The concentration of each hormone in saline was kept constant for each injection (ACTH, 0.83 mg/ml; Synacthen, 0.31 mg/ml; and prolactin, 2.50 mg/ml).

Preparation of Gamma-Globulin

Blood was allowed to clot, refrigerated over night, centrifuged, and the serum was removed with a Pasteur pipette. Merthiolate (1:10,000) was added as a preservative and the serum was stored by freezing small samples in serum bottles. The gamma-globulin was separated from the serum by adding an equal volume of a saturated solution of ammonium sulphate, adjusting the *p*H to 7.8 with 1.0 N NaOH, and subsequent centrifugation. The last procedure was repeated twice except that the final precipitate was dissolved in borate-buffered saline (*p*H 8.4) of half the original serum volume. The gamma-globulin was dialyzed in borate-buffered saline at 4 C until no more sulphate was found in the dialyzate when tested with barium chloride. The gamma-globulin was stored frozen in shell vials.

Estimation of Titer and Determination of Specificity

Anti-ACTH and anti-Synacthen titers were estimated by haemagglutination since Leznoff et al. (1962) found that no precipitation occurs between ACTH and anti-ACTH serum in ring tests or in gel. Washed sheep or rabbit erythrocytes were treated with tannic acid and then coated with the antigen. Serial two-fold dilutions of the antiserum sample were prepared and a constant volume of the coated erythrocytes was added to each of them. The settling patterns were observed for agglutination so that the titer could be obtained. Anti-prolactin titers were determined by haemagglutination as well as interfacial ring-tests.

The specificity of each of the three antisera was tested by haemagglutination and Ouchterlony gel diffusion with ACTH, Synacthen, prolactin, luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and growth hormone. A separate series of haemagglutination tests was conducted to study the cross reaction between each of the prepared antisera and pituitary homogenates of chinook salmon (*Oncorhynchus tshawytscha*) (see Table 2).

Table 2. Immunochemical tests for specificity of the antisera.

		Antigen[a]							
Test	Antibody	ACTH	Syn.	LTH	TSH	FSH	LH	GH	Homogenate
Gel diffusion	Anti-ACTH	−	−	−	−	−	−	−	−
	Anti-Syn.	−	−	−	−	−	−	−	−
	Anti-LTH	−	−	++	−	−	−	−	−
Haemagglutination	Anti-ACTH	++	+	−	−	−	−	−	+
	Anti-Syn.	+	+	−	−	−	−	−	±
	Anti-LTH	−	−	++	−	−	−	−	+

[a] ACTH = adrenocorticotrophic hormone (Porcine, Sigma); Syn. = Synacthen ($\beta^{1\text{-}24}$-corticotrophin, Ciba); LTH = prolactin (Ovine, Sigma); TSH = thyroid-stimulating hormone (Bovine, Sigma); FSH = follicle-stimulating hormone (Porcine, Sigma); LH = luteinizing hormone (Equine, Calbiochem.); GH = growth hormone (Porcine, Sigma); Homogenate = adult chinook salmon pituitary glands in saline; ++ = strong reaction; + = weak reaction; − = no reaction.

Preparation of Gamma-Globulin with Fluorescing Dye

The protein percentage of the gamma-globulin fraction was estimated with the Biuret method and then changed to a 1.0% protein solution by adding borate-buffered saline (*p*H 8.4). The gamma-globulin solution was placed in a dialysis bag in a graduated cylinder mounted on a magnetic stirrer in the refrigerator. A solution of carbonate–bicarbonate buffer (*p*H 9.0) containing 0.1 mg/ml of fluorescein isothiocyanate (FITC) (10 v/v of gamma-globulin solution) was placed in the cylinder and labelling was allowed to proceed from 16 to 24 hr (Clark and Shepard, 1963). At the end of this period, the unconjugated dye was removed by gel filtration through a column (1 × 15 cm) of Sephadex G-50 beads equilibrated with phosphate-buffered saline (0.01 M phosphate, 0.15 M NaCl, *p*H 7.3). From every 2-ml fluorescent gamma-globulin solution, 20 fractions of 2 ml each were collected. This was found adequate to rid the globulin solution of unbound dye.

It is necessary to realize that the conjugated protein solution may well contain a variety of globulins. Moreover, the ratio globulin–FITC probably varies and evidence has been presented that adequate staining with the antibody depends on this ratio. Too much dye would convert the antibody into an unspecific acid dye; too little dye would lead to an insufficiently fluorescing final product (Mayersbach, 1967). Therefore, the dye-free gamma-globulin fractions were further fractionated by placing them in a column (1 × 15 cm) of DEAE Sephadex and eluting them stepwise with NaCl (0.15 M, 0.20 M, 0.30 M, 1.0 M, 2.0 M) in 0.01 M phosphate buffer (*p*H 7.3). From every 2 ml added to the column, 10 fractions of 2 ml each were obtained and the desired specific conjugate selected.

COLLECTION OF PITUITARY GLANDS

Sockeye salmon belonging to the "Chilco race" were collected at four locations along their migratory route. One sample (group 1) was taken from sea water near Lummi Island, approximately 30 miles from the mouth of the Fraser River; two others (groups 2 and 3) were river fish collected at 40 and 200 miles upstream respectively. The last sample (group 4) consisted of sexually mature fish, captured on the spawning grounds in Chilco Lake. Scale samples were taken to verify that all fish were of the same race. There were at least eight fish in each sample. Four pituitaries were immediately frozen in isopentane-liquid nitrogen and kept frozen in sealed vials. Several others were fixed in Bouin Hollande-Sublimate (B.H.S.). In addition, to prepare pituitary homogenates, 110 glands were collected from sexually mature chinook salmon at the Big Qualicum River on Vancouver Island. These glands were frozen on dry ice.

HISTOLOGICAL TECHNIQUES

The pituitaries kept in isopentane were sectioned in a cryostat at −18 C. Sections were received on cover slips and allowed to dry. Sections to be reacted with anti-prolactin were fixed in 95% ethanol. Since Emmart et al. (1965) state that prolactin is readily extracted by alcohol fixation, time was limited to 1.5 min at room temperature. Sections to be treated with anti-ACTH or anti-Synacthen were fixed for 30 min at room temperature in 70% ethanol. After fixation the sections were briefly rinsed in distilled water, air dried, and incubated in the antibody solution for 30 min at room temperature. A hanging droplet technique was used, the cover slips being kept in small sealed petri dishes. After incubation the sections were briefly rinsed in phosphate-buffered saline (*p*H 7.3), then placed in the same solution for 2 hr (two changes) to wash off excess fluorescent antibodies. Subsequently, the sections were rinsed and mounted in distilled water and viewed with a fluorescent microscope (Carl Zeiss, Universal). After photographing the fluorescence the illumination was switched to phase contrast and the same field rephotographed. The cover slips were then floated off, the sections fixed in B.H.S. and stained with one of the following methods: Masson's trichrome, periodic acid-Schiff (PAS), or hematoxylin–erythrosin–orange G–aniline blue. In addition to the direct method mentioned above, other sections were subjected to the indirect staining technique. This involved reacting the sections first with the specific unlabelled antibodies and then with fluorescein-labelled anti-rabbit gamma-globulin. Three types of control reactions were carried out: unlabelled antibody was applied to sections to determine if it would block the fluorescein labelled antibody; labelled normal rabbit gamma-globulin was applied to determine if there were any reactions; untreated sections were checked for the presence of native fluorescence.

The pituitary glands fixed in B.H.S. were embedded in paraffin, sectioned serially at 5 μ and stained with Masson's trichrome method. Sections thus prepared were used to examine the cell types which reacted positively to treatment with the antibody solutions. The granulation in these cells was analyzed with a microspectrophotometer (Shimadzu, Model MPS-50L). These cells stain with xylidine ponceau and thus, twofold serial dilutions of this dye were used to determine the wavelength of maximum absorbance and to ascertain whether or not this dye solution followed the Lambert–Beer law. Fifty measurements were taken of each fish by scanning 50 cells with a 5-μ aperture and recording the maximum absorbance of each of these cells.

RESULTS

IMMUNOCHEMICAL TESTS

Considerable differences were found between individual rabbits with respect to the immunization response. Irrespective of the antigen used, the variation concerned the onset of antibody formation as well as the maximum titer. Therefore, and also since it has been suggested that different animals may produce antibodies of different quality (Fleischer et al., 1966), sera were not pooled and only those of the highest titer were used.

Antiserum to ACTH reacted not only with ACTH but also cross-reacted with Synacthen. Anti-Synacthen showed a similar behaviour in that it reacted with both of these antigens. The reaction, anti-Synacthen-Synacthen, was much weaker than that of anti-ACTH-ACTH. Also the relevant cross-reactions were only weakly positive. Ouchterlony gel diffusion tests showed that antiserum to prolactin reacted only with the homologous antigen and failed to produce a precipitate with any other hormone (Fig. 1). Two precipitation bands developed, which is probably due to the occurrence of prolactin polymers (Emmart et al., 1965). In haemagglutination tests, similarly, this antiserum reacted exclusively with prolactin. When tested against a chinook pituitary homogenate, all of the three antisera failed to give positive precipitation results. With the haemagglutination test, a weak reaction was observed. Table 2 summarizes the results.

HISTOCHEMICAL RESULTS

Anti-ACTH was bound invariably in a restricted area, located near the pars nervosa, separating this part from the remainder of the rostral pars distalis (Fig. 7). After staining of sections with Masson's trichrome, this area appeared to consist of cells assuming an orange–brown colour. These cells have been identified as ***epsilon*** cells (van Overbeeke and McBride, 1967). Fluorescence in the epsilon cells was confined to and evenly distributed throughout the cytoplasm, but further localization was impossible. In Masson's trichrome-stained sections, granules were hardly discernable. Essentially the same results were obtained with anti-Synacthen, except that the fluorescence was much weaker. In both instances, pre-treatment with the unlabelled homologous antibody solution completely blocked the reaction.

Incubation of sections with anti-prolactin produced a yellow–green fluorescence in the rostral pars distalis. Identification of the fluorescence showed that the antibody had been bound specifically to the follicularly arranged *eta* cells. Nonfluorescing cells, either isolated or in small groups, were found to be PAS-positive cells (Fig. 3). Similarly, no fluorescence was present in blood vessels (Fig. 2). Limited resolution and the presence of some free dye impeded precise localization of the fluorescence in the cytoplasm of the eta cells. However, there was sufficient evidence that it was bound to the secretory granules. First, the juxtaluminar part of the cells stained negatively (Fig. 5) and examination of Masson's trichrome-stained sections showed that in these parts there are no granules. Moreover, we found a clear positive correlation between the intensity of the fluorescence throughout the rostral pars distalis and the degree of cytoplasmic granulation (Fig. 6). No fluorescence was seen in the lumina of the follicles (Fig. 5). The reaction described above could be totally prevented by blocking the binding sites first with unlabelled anti-prolactin.

Epsilon cells as well as eta cells could also be identified with the indirect method but the fluorescence was weaker than if the direct method were applied.

Reaction with labelled normal rabbit antibodies produced no fluorescence. The sections did not exhibit native fluorescence.

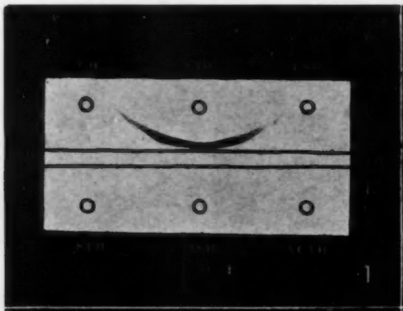
CHANGES DURING MIGRATION

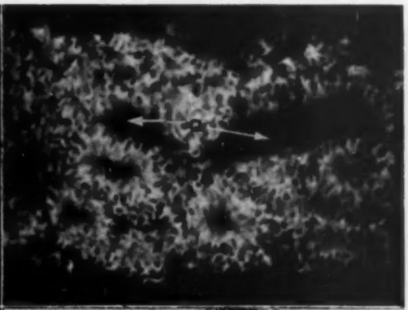
Since it is difficult to standardize the fluorescent staining, no attempt was made to quantitatively evaluate the results obtained with the various samples of fish. Therefore, Masson's trichrome-stained sections were used to investigate whether there were any secretory activity changes during migration. Comparisons of the various groups of fish, with respect to the density of granulation in the epsilon cells, showed that there was an increase in the later stage of the migration (Fig. 13). However, the density of the cytoplasmic stain in the eta cells remained unaltered (Fig. 14).

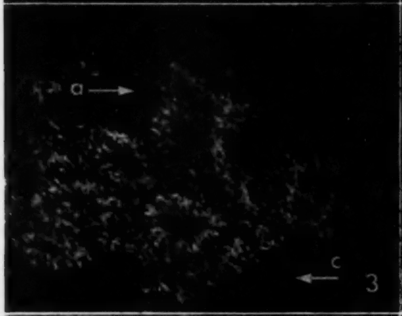
DISCUSSION

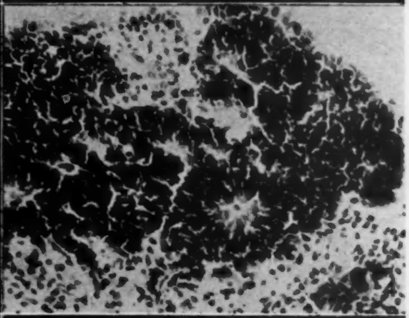
Of all the pituitary cell types in the sockeye salmon, only the epsilon cells reacted with anti-ACTH. This is in agreement with findings by Olivereau and Ball (1963, 1964, 1965), Olivereau (1965), Ball et al. (1965), and Ball and Olivereau (1966) who, by using histophysiological techniques in other teleosts, were able to identify these cells as ACTH cells.

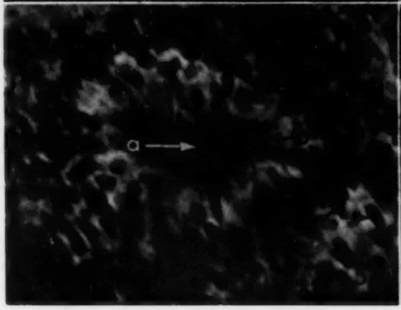
Immunohistochemical studies with ACTH in mammals demonstrated that heterologous cross reactions can be obtained. Leznoff et al. (1962) showed that anti-porcine ACTH cross-reacted with human ACTH. The same conclusion can be inferred from the studies by Fleischer et al. (1966) although these investigators observed that some sera were species specific. So far, to our knowledge, no immunological similarities have been shown between mammalian and fish ACTH. It is interesting that in our studies positive reactions were obtained, not only with anti-mammalian ACTH, but also with antisera to the synthetic analogue, Synacthen (β^{1-24}-corticotropin). This latter reaction was much weaker, probably due to the low titer.

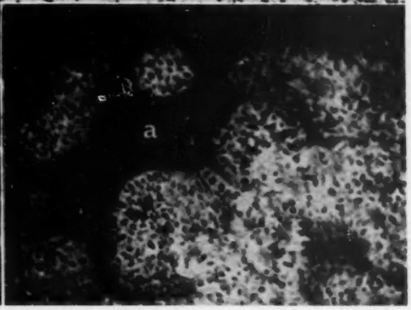












All photographs were taken with Ilford FP3 film.

FIG. 1. Cell diffusion test. Antiserum to prolactin (LTH) tested for specificity with six pituitary hormone solutions: LH (luteinizing hormone), FSH (follicle-stimulating hormone), STH (somatotropic hormone), TSH (thyroid-stimulating hormone), and ACTH (adrenocorticotropic hormone). Each well contained the antigen in a concentration of 1.0 mg/ml. The antiserum reacted only with prolactin.

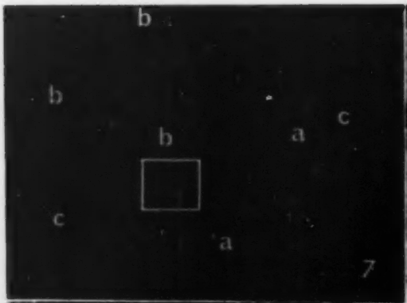
FIG. 2. Distal pars distalis of the pituitary gland of a sockeye caught at the spawning grounds. The section was stained with fluorescent antiprolactin, which became specifically bound to the eta cells. Blood vessels (*a*) did not stain. ×125.

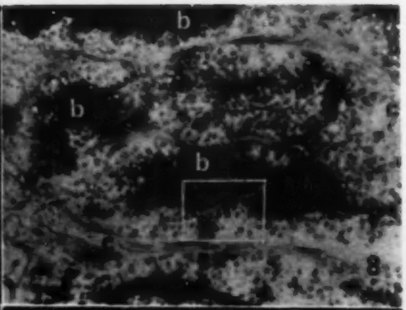
FIG. 3. Pituitary of seawater sockeye stained with fluorescent antiprolactin showing fluorescence of the eta cells of the distal pars distalis. The eta cells appear follicularly arranged (*b*). The basophil cells of the distal pars distalis (*a*) as well as the cells of the proximal pars distalis (*c*) did not bind the dye. ×120. Compare with Fig. 4.

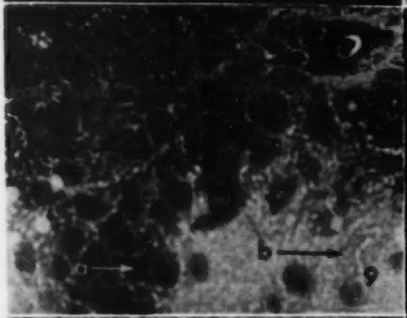
FIG. 4. Section adjacent to the one in Fig. 3 stained with the Masson's trichrome method, to identify the fluorescing cells in Fig. 3. Only the eta cells are well stained with this technique. Bright-field illumination. ×130.

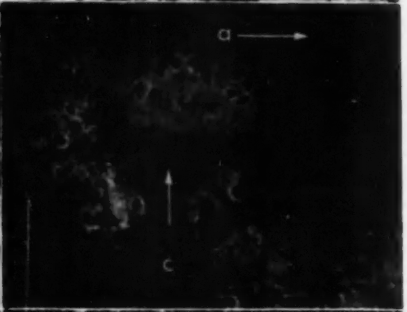
FIG. 5. Higher magnification of follicle *b* in Fig. 3. Note that the fluorescence is most intense in the basal parts of the cells (*b*), only weak in the apical portion, and absent from the follicle lumen (*a*). The nuclei do not react with antiprolactin. ×390.

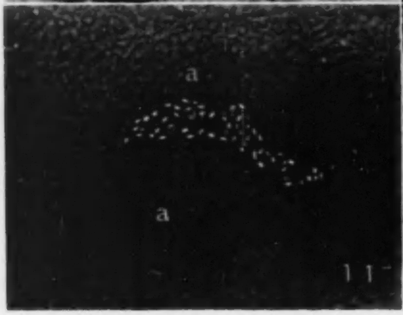
FIG. 6. Pituitary of sockeye caught at the spawning grounds. Stained with antiprolactin. Area of the distal pars distalis near the pars nervosa (*a*). Note that all eta cells fluoresce but that the intensity varies. ×130.











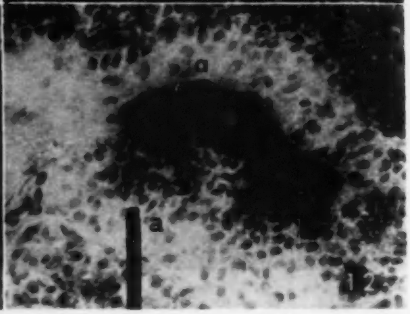


FIG. 7. Pituitary of sockeye which had just entered fresh water. The section was stained with fluorescent ACTH. Only epsilon cells (*a*) showed a positive reaction. Most of the cells that did not react are prolactin cells (*b*). Similarly, small protrusions of the pars nervosa did not bind the dye (*c*). ×105.

FIG. 8. Same section and identical area as shown in Fig. 7, after staining with Masson's trichrome. Labelling corresponds to that in Fig. 7. Note that the epsilon cells (*a*) stained weakly as compared with the prolactin cells (*b*). Bright-field illumination. ×115.

FIG. 9. Higher magnification of the area indicated in Fig. 7 and 8. Masson's trichrome method stained the prolactin cells deep red (*a*) whereas the epsilon cells assumed a characteristic yellowish-brown colour (*b*). Bright-field illumination. ×460.

FIG. 10. Pituitary of sockeye caught at the spawning grounds, stained with fluorescent anti-ACTH. Distinct fluorescence is seen in the epsilon cells, some of which show the typical columnar shape (*b*). Prolactin cells (*a*) and pars nervosa (*c*) are unstained. ×310.

FIG. 11. Sockeye pituitary gland stained with fluorescent anti-ACTH. Pointer shows location of fluorescing cells (*a*). Photograph taken with phase contrast illumination. ×210. Compare with Fig. 12.

FIG. 12. Same section as shown in Fig. 11, stained with Masson's trichrome in order to identify the fluorescing cells (*a*). Bright-field illumination. ×210.

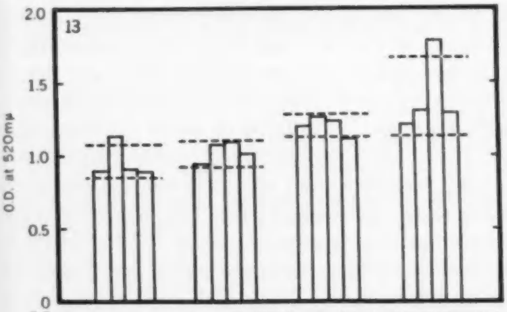


FIG. 13. Microspectrophotometric analysis of granule density in epsilon cells. Each bar represents the mean of 50 readings of the cells in one fish. Broken lines indicate the standard errors for each group of four fish. Each of the means for groups 1 and 2 differ significantly from each of the means for groups 3 and 4 ($P < 0.025$).

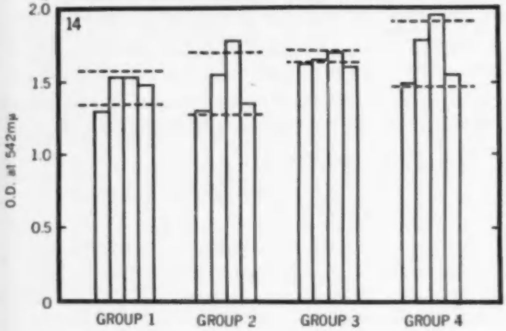


FIG. 14. Microspectrophotometric analysis of granule density in eta cells. Each bar represents the mean of 50 readings of the cells in one fish. Broken lines indicate the standard errors for each group of four fish. The groups do not differ significantly.

Synthetic ACTH was introduced as an antigen by Kracht et al. (1965) because of its lack of contaminants. Kracht et al. (1965, 1966) and Hachmeister and Kracht (1965) showed that anti-$\beta^{1\text{-}24}$-corticotropin was bound in the ACTH cells of the human, dog, cat, and rabbit. Fleischer et al. (1966) also showed that ACTH and a synthetic analogue ($\alpha^{1\text{-}24}$-corticotropin) possess the same immunological properties.

During migration, the ACTH cells did not exhibit any appreciable changes in cellular or nuclear hypertrophy. But there was an increase in the amount of secretory granules (Fig. 13). Robertson and co-workers have shown that in chinook salmon the adrenal cortex undergoes considerable hypertrophy during the spawning migration (Robertson and Wexler, 1957, 1959). Although we did not examine the adrenal cortex in this study, previous work indicated a similar phenomenon in sockeye salmon (A.P. van Overbeeke, unpublished data). It is, therefore, very likely, that the change in the ACTH cells is to be related to the adrenocortical hypertrophy.

The specific binding of anti-ovine prolactin in the eta cells of the sockeye pituitary gland is in complete agreement with the results obtained by Emmart et al. (1966) and Emmart and Mossakowski (1967) in their study of the pituitary gland of *Fundulus heteroclitus*. Our results, furthermore, confirm the investigations by Ball and Olivereau (1964) and Ball (1965a, b, c), who first identified this cell type as the source of teleost prolactin. In some euryhaline species of teleosts, these cells exhibit changes indicative of enhanced secretory activity when the fish under experimental conditions are transferred from salt water to fresh water: *Fundulus heteroclitus* (Ball and Pickford, 1964; Ball and Olivereau, 1964, 1965; Olivereau and Ball, 1964); *Tilapia mossambica* (Dharmamba and Nishioka, 1968). These species and some others lose the ability to survive in fresh water after hypophysectomy, but treatment with mammalian prolactin may reverse this response (Pickford and Phillips, 1959; Ball and Olivereau, 1964; Handin et al., 1964; Schreibman and Kallman, 1964, 1966). Whether prolactin is also required for survival in fresh water in sockeye salmon remains to be investigated. The absence of cytological changes in the eta cells during entrance of the fish into the river, noted in the present study and in an electron microscopical investigation by Cook and van Overbeeke (1969), could suggest that the role of prolactin in sockeye differs from that in other euryhaline teleosts. It is also possible, however, that under natural conditions of anadromous migration, changes in the physiology of the prolactin cells are less drastic and do not alter their cytomorphological features.

ACKNOWLEDGMENTS

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Some Physiological Consequences of Aortic Catheterization in the Brook Trout (*Salvelinus fontinalis*)[1]

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HOUSTON, A. H., MARY ANNE DEWILDE, AND JANE A. MADDEN. 1969. Some physiological consequences of aortic catheterization in the brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Canada 26: 1847–1856.

The effects of aortic catheterization upon the hematology and water-electrolyte regulation of brook trout have been followed for a period of 72 hr after completion of the procedure. The data indicate the markedly traumatic character of techniques of this general type, and the need for much longer recovery periods than have generally been allowed in studies on the cardiovascular physiology of salmonids.

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INTRODUCTION

THE DEVELOPMENT OF TECHNIQUES for the implantation of vascular catheters in teleost fishes (Labat and Laffont, 1964; Hickman, 1965; Smith and Bell, 1964, 1967) has made possible the investigation of a variety of problems relating to cardiovascular function in these animals. With the exception of work by Randall et al. (1965), however, surprisingly little attention has been directed toward assessment of the physiological consequences of this type of intervention. Survey of recent literature in the general area suggests that such procedures have apparently been widely assumed to be essentially nontraumatic in character. Accordingly, specimens so prepared have been experimentally employed immediately upon recovery from anesthetization, or within a few hours thereafter.

A number of observations made during the course of studies upon thermoacclimatory variations in the hematology and blood volume of trout (Houston and DeWilde, 1969) suggested that this assumption might be open to question, and, indeed, that catheterization induces significant modifications in body fluid composition. Similar conclusions have been reached by Rahn (personal communication) as a consequence of work upon acid-base balance in carp, and are, of course, consistent with the findings of earlier studies upon the effects of experimental handling on fishes (Meyer, 1948; Forster and Berglund, 1956). The present investigation was, accordingly, undertaken in an attempt to define some of the effects induced by such procedures, and

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to estimate the interval required for restoration of "normal" status in the animals. Because of the specific interests of this laboratory the emphasis of the study was directed toward possible modifications in the water-electrolyte regulation and hematology of a representative salmonid, the brook trout, *Salvelinus fontinalis*.

The basic protocol of the investigation involved initial implantation of dorsal aortic catheters, and the immediate withdrawal of samples for determination of normal values. Groups of up to five animals were then killed at intervals (3, 6, 24, 48, 72 hr) subsequent to completion of the procedure; three series of fishes being used. No attempt was made to prolong sampling beyond 72 hr since, in practice, little success has as yet attended our efforts to maintain aortic catheters patent for more than about 2 days. In most instances initial values were obtained for red cell numbers, hematocrit and hemoglobin content. In addition, to the extent that sample volumes permitted, estimates of plasma sodium, potassium, and chloride were carried out. Upon termination of each experiment all analyses were repeated, and determinations of muscle water, sodium, potassium, and chloride made. To amplify the control data an additional group of trout were catheterized and blood, plasma, and tissue samples taken immediately. Values derived from these animals were not statistically distinct from the blood and plasma values obtained during the main study.

MATERIALS AND METHODS

EXPERIMENTAL STOCKS

Two-year old specimens were purchased from a local commercial hatchery and varied in weight from 186.3 to 433.8 g (312.1 ± 12.3 g). The animals were maintained under conditions essentially similar to those described in earlier studies upon rainbow trout (Houston et al., 1968) at temperatures ranging from 5 to 8 C. All remained in active and apparently healthy condition under these circumstances during the course of the study.

AORTIC CATHETERIZATION

Catheter placement was carried out by methods similar to those described by Smith and Bell (1967) and Holeton and Randall (1967). A short piece of flared PE-160 tubing was passed through a hole punched in the snout central to the right nasal aperture to act as a support for the catheter proper. The latter consisted of a length of PE-50 tubing equivalent to 0.1 ml in volume (i.e., 35–40 cm) to which was attached a 15-mm 23-guage deflected (Huber) point needle. After insertion of the needle into the aorta the catheter was sutured to the roof of the mouth, and anchored within the nasal tube with two ligatures. The initially drawn sample was replaced by an equivalent volume of sterile, heparinized Courtland saline, less glucose (Wolf, 1963) and the catheter filled and plugged.

The traumatic consequences of the procedure appeared to be minimized, and the recovery period reduced under conditions which provided for rapid work upon minimally anesthetized and well-irrigated animals.[2] With the arrangement indicated in Fig. 1 the procedure could normally be completed in 3–6 min upon specimens previously anesthetized by immersion for 3 min in 0.15% aqueous tricaine methanesulphonate ("Finquel," Ayerst). The animals were then irrigated, if necessary, until the spontaneous initiation of ventilatory movements, and trans-

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[2] Subsequent work has indicated that irrigation with oxygenated isotonic saline improves recovery, and reduces trauma to some extent.

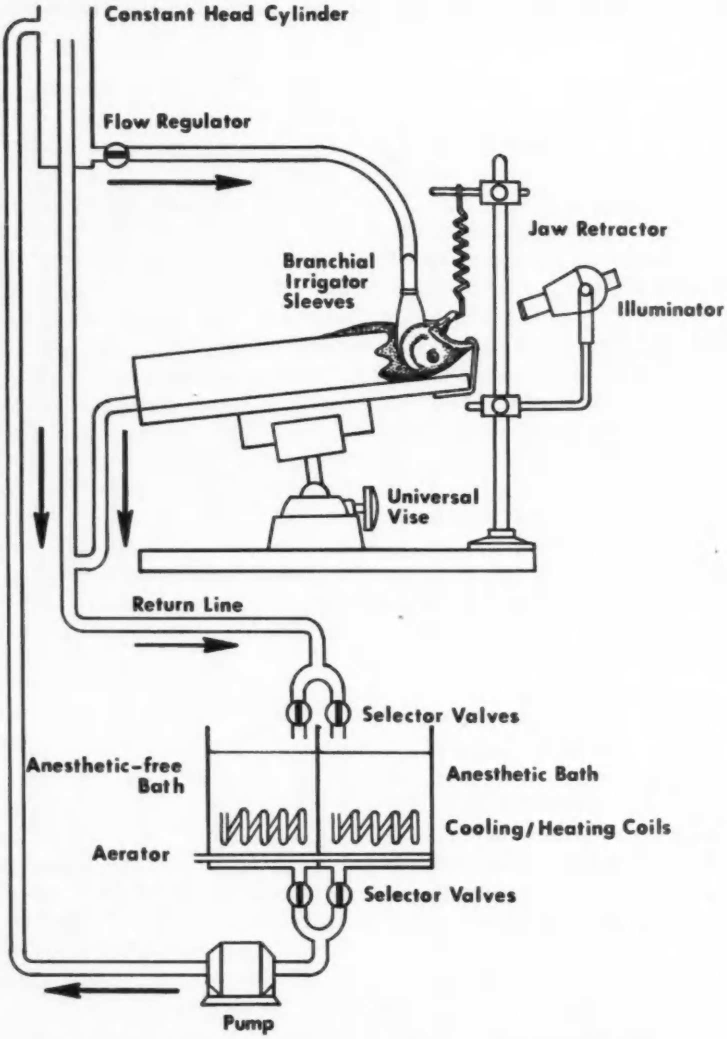
Constant Head Cylinder
Flow Regulator
Jaw Retractor
Branchial Irrigator Sleeves
Illuminator
Universal Vise
Return Line
Selector Valves
Anesthetic-free Bath
Anesthetic Bath
Cooling/Heating Coils
Aerator
Selector Valves
Pump

FIG. 1. General arrangement of system used for catheter implantation.

ferred to small, circular aquaria suspended in their original acclimation tanks. Recovery from anesthesia, as judged by normalcy of swimming movements, was usually completed within 10–30 min.

SAMPLING AND ANALYSIS

Sampling techniques, and blood, plasma, and tissue analytical methods were similar to those described in earlier reports (DeWilde and Houston, 1967; Houston and DeWilde, 1968; Houston et al., 1968), as were the procedures used for data evaluation.

RESULTS

HEMATOLOGY

Mean values for the hematological characteristics of the experimental population (erythrocyte number = 1.39 ± 0.03 × 10^6 cells/mm^3; hematocrit = 37.0 ± 0.5%; hemoglobin = 8.4 ± 0.5 g/100 ml) were in reasonably good agreement with those previously reported for the salmonids. All three displayed marked change during the immediately post-procedural interval (Fig. 2), a sharp decline in absolute value being coupled with a greater or lesser increase in sample variability. Thus, erythrocyte numbers dropped to 0.78 ± 0.14 × 10^6 cells/mm^3, packed cell volume to 23.4 ± 4.2% and hemoglobin to 5.6 ± 0.8 g/100 ml after 3 hr. Substantial recovery, as indicated by subsequent increases to near-normal values, was apparent after 24 hr. Although all hematological parameters remained from 5 to 10% below their original levels throughout the 72-hr observation period, this may have represented little more than a dilution effect. The volume of blood initially removed and replaced by saline represented approximately the same percentage of the total blood volume typical of this species at the temperatures employed (Houston and DeWilde, 1969).

Also included in Fig. 2 are data derived from an exploratory series in which the duration of the procedure was deliberately extended, and the fish handled less gently than was normally the case. As can be seen a much more marked departure from the normal state was induced, whereas the period of recovery was greatly extended.

WATER-ELECTROLYTE BALANCE

Plasma electrolyte levels were also comparable with those observed in earlier studies upon this group of fishes (e.g., Holmes and McBean, 1963; Hickman et al., 1964; Holmes and Stainer, 1966; Houston et al., 1968). A more varied picture of response to catheterization was encountered with respect to ionic regulation than had been the case for the various hematological parameters. Thus, whereas potassium levels tended to remain fairly stable, plasma chloride dropped from 128.0 ± 1.6 mM/liter to 123.0 ± 3.2 mM/liter after 3 hr, and to 121.4 ± 3.9 mM/liter at the 6-hr sampling period. Some increase in concentration was apparent after 24 hr, the situation, in general, paralleling that seen with respect to erythrocyte levels, hematocrit and hemoglobin concentration. By contrast, plasma sodium tended to rise after catheterization, increasing from 123 ± 3 mM/liter to 133 ± 5, 129 ± 4, and 141 ± 3 mM/liter after 24, 48, and 72 hr respectively. Because of this, rather marked variations in the chloride:sodium plus potassium ratio were observed. Assuming the principle of overall body fluid electroneutrality to hold, such changes suggest the likelihood of compensatory modifications in some anion or cation level. The most common ionic variant under such circumstances is bicarbonate. Accordingly these data suggest the possibility of coupled disturbances in water-electrolyte and acid–base regulation, an hypothesis verified by recent findings on carp (Rahn, personal communication).

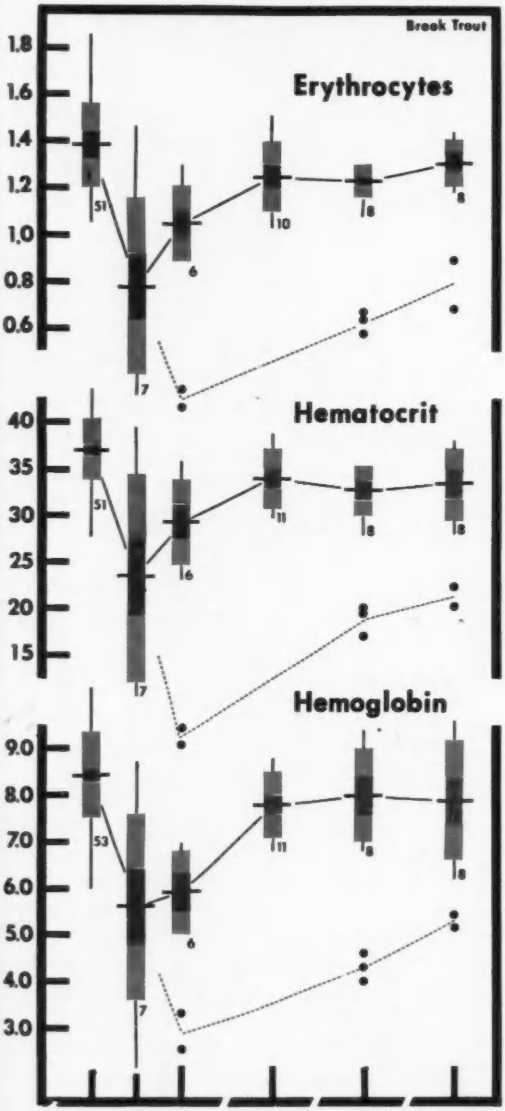

FIG. 2. Modifications in the hematology of the brook trout subsequent to dorsal aortic catheterization. Ordinate: time in hours after completion of procedure. Abscissa: erythrocytes (cells × 10^6/mm^3), hematocrit (%), and hemoglobin (g/100 ml); vertical line — range; vertical hatched bar — one standard deviation of the mean; vertical dotted bar — one standard error of the mean; horizontal bar — sample mean.

The tissue electrolytes, chloride and potassium, showed a mutually consistent pattern of change in the sense that both dropped sharply after catheterization (Fig. 4). Chloride, for example, fell from a mean of 10.8 ± 0.5 mM/kg to 8.6 ± 0.4 mM/kg after 3 hr, and displayed no appreciable increase

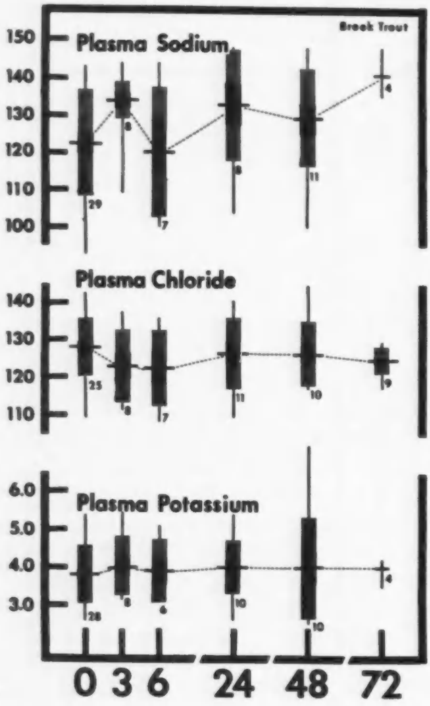

FIG. 3. Modifications in the plasma electrolyte composition of the brook trout subsequent to dorsal aortic catheterization. Ordinate: time in hours after completion of procedure. Abscissa: plasma sodium (mM/liter), plasma chloride (mM/liter), and plasma potassium (mM/liter); vertical line — range; vertical hatched bar — one standard deviation of the mean; vertical dotted bar — one standard error of the mean; horizontal bar — sample mean.

prior to the 72-hr sampling period. Potassium declined even more dramatically, falling by some 40% from the original value of 65.6 ± 2.4 mM/kg over the same interval. Recovery was, however, much more rapid than had been the case with chloride. A procedural error demanded rejection of all sodium values for the control group of animals. The remaining data did, nevertheless, suggest that the pattern of post-procedural variation might differ from that seen for both tissue chloride and potassium levels. In this instance there was some suggestion of a transient increase followed by a drop to near-normal values after 48 hr.

Finally, tissue water content rose significantly after completion of catheterization, increasing from 751.5 ± 3.6 g/kg to 763.8 ± 2.1 g/kg after 6 hr (Fig. 5). This rise in hydration level was prolonged in character, with normal values for tissue water being achieved only after a lapse of 72 hr. Approximate partitioning of overall water content by the "chloride space" technique suggested that the entire water increment was localized in the cellular compartment. This, in turn, suggests the probability of factors prompting significant volume transfers from the extracellular to the cellular phase.

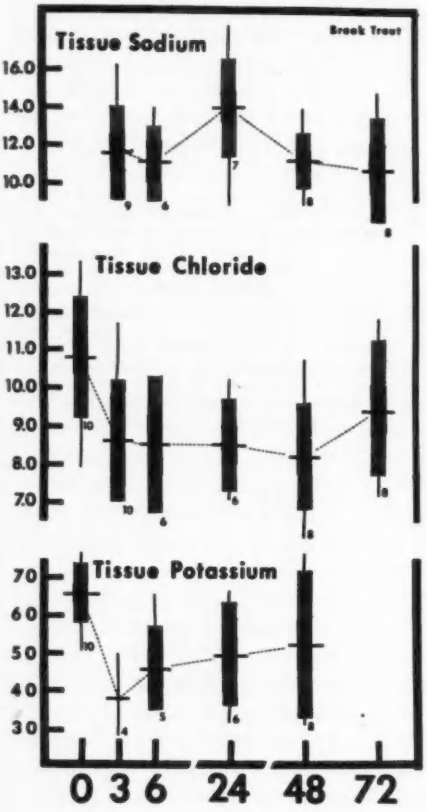

FIG. 4. Modifications in the tissue electrolyte composition of the brook trout subsequent to dorsal aortic catheterization. Ordinate: time in hours after completion of procedure. Abscissa: tissue sodium (mM/kg), tissue chloride (mM/kg), and tissue potassium (mM/kg); vertical line — range; vertical hatched bar — one standard deviation of the mean; vertical dotted bar — one standard error of the mean; horizontal bar — sample mean.

DISCUSSION

The data obtained in the present study clearly indicate the traumatic consequences of experimental interventions of the general class represented by the aortic catheterization procedure. This finding was not unexpected. Forster and Berglund (1956), for example, reported a marked "laboratory diuresis" in marine fishes subjected to handling. Although it has generally been assumed that freshwater species are less sensitive than marine forms in this regard, Hickman (1965) has demonstrated sharp transient increases in urine flow and urinary electrolyte loss in the white sucker after cannulation of the urinary bladder, a procedure which, in terms of handling and anesthetization, is similar to vascular catheterization. Comparable findings have also been reported by J. B. Hunn (personal communication), and are consistent with our observations upon kidney function in carp (Madden and Houston, unpublished data).

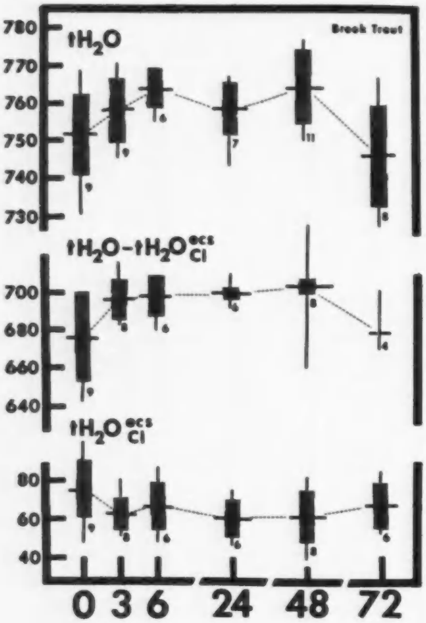

FIG. 5. Modifications in the tissue water content and distribution of the brook subsequent to dorsal aortic catheterization. Ordinate: time in hours after completion or procedure. Abscissa: tissue water (g/kg), "cellular phase volume" (tissue water content–chloride space, g/kg), "extracellular phase volume" (chloride space, g/kg); vertical line — range; vertical hatched bar — one standard deviation of the mean; vertical dotted bar — one standard error of the mean; horizontal bar — sample mean.

The rapid onset, and the duration of the effects observed, have clear implications with respect to the use of specimens prepared in this fashion for subsequent experimentation. Full recovery, as judged from temporal variations in plasma and tissue composition, may well require several days with the techniques now in use. Against this must be balanced the practical difficulties associated with maintaining such catheters in position and patent over sufficient periods of time. It is apparent, however, that recovery periods of much longer duration than have thus far been allowed should be employed. There is, indeed, some basis for the supposition that a significant proportion of the now-available information upon cardiovascular function in fishes may well have been obtained from specimens exhibiting close to maximal deviation from the physiologically "normal" state.

Although the basis of the effects observed is undoubtedly complex, a number, at least, are consistent with the concept of increased endosmosis and electrolyte efflux, i.e., the noted decreases in plasma chloride, tissue potassium and chloride, and "extracellular" phase volume, and rising values for tissue water content and "cellular" volume. That an increase in endosmosis actually takes place is further substantiated by Hickman's observation (Hickman, 1965) that urine flow in suckers increases by several hundred per cent after cannulation of the urinary bladder. Increases in branchial electrolyte efflux also follow handling (Meyer, 1948), and this mode of loss is, of course, magnified by that which unavoidably accompanies increased renal activity.

The hypothesis that such effects may represent the consequences of changes in branchial water-electrolyte permeability cannot be ruled out, particularly in view of the work of Gordon (1963). Equally plausible, however, is the suggestion that they may stem from reflex adjustments in cardiac output and branchial exchange surface area. Both factors have been implicated in the amplification of branchial gas exchange, and appear to be increased in response to vascular hypoxia (Taylor et al., 1968). The latter condition almost certainly obtains during operative work despite irrigation, although no data on this point appears in the literature. Increases in ventilatory and cardiac activity during recovery (Serfaty et al., 1959; Randall, 1962) might then prompt the hypothesized changes in branchial water-electrolyte fluxes even in the absence of permeability variations.

Although some speculative basis for the compositional changes observed can be provided, the mechanisms suggested do not account for the marked and immediate changes seen in the hematological status of the catheterized specimens. It is of some interest that the blood samples taken from such animals showed little evidence of hemolytic effects, and that the kinetics of red cell formation in fishes are seemingly insufficient to account for the degree of recovery observed within the initial 24-hr period. It is, however, possible that substantial quantities of erythrocytes may have been transiently isolated in capacitance areas of the vascular system. Autopsies were not routinely performed upon killed specimens. However, in some instances there was evidence of blood pooling in tissue areas posterior to the branchial chamber. It may be noted that the observations of Randall et al. (1965) upon blood pressure changes in rainbow trout during recovery from M.S. 222 anesthesia are not inconsistent with this suggestion. The findings of the latter study taken in conjunction with those reported herein clearly point to the need for further investigation of methods for minimizing the undesirable sequelae of this otherwise highly valuable method of study.

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Lipolytic Activity Toward Long-Chain Triglycerides in Lateral Line Muscle of Rainbow Trout (*Salmo gairdneri*)[1]

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BILINSKI, E., AND Y. C. LAU. 1969. Lipolytic activity toward long-chain triglycerides in lateral line muscle of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada 26: 1857–1866.

Lipolytic activity toward long-chain triglycerides was studied in the lateral line muscle of rainbow trout (*Salmo gairdneri*) by use of tissue slices. Emulsification of substrates with phospholipids was found to be necessary to demonstrate lipolysis, which occurred in decreasing intensity with tripalmitin, triolein, and tristearin. The lipolytic activity showed optimum activity at *p*H 7.3 and it was inhibited by NaF, protamine, and *p*-chloromercuriphenyl sulfonate. Albumin had little effect on the activity. Epinephrine did not stimulate lipolysis, but it reduced loss of activity during preincubation.

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INTRODUCTION

FISH MUSCLE is known to contain a lipase able to catalyze the hydrolysis of short chain triglycerides (Wood, 1959a, b; Santa and Bottesch, 1961; George, 1962), but the presence of lipolytic activity toward long-chain triglycerides does not appear to be well established in this tissue. A higher lipolytic activity was found in the red than in the white muscle of fish and this property, considered in conjunction with other biochemical differences, led George to observe that the red muscle is better adapted to utilize fat as a source of energy (George, 1962; George and Bokdawala, 1964). This interpretation is also supported by much higher capacity for fatty acid oxidation of the lateral line (red) muscle in salmonid fish (Bilinski, 1963; Bilinski and Jonas, 1964; Jonas and Bilinski, 1964) and by the observations made on the depletion of fat from red muscle during swimming in dogfish (Bone, 1966). The present understanding of the metabolic processes involved in the lipid catabolism in fish muscle is reviewed elsewhere (Bilinski, 1968).

In the present study, the lipolytic activity in the lateral line muscle of rainbow trout was investigated in relation to its physiological function in promoting the mobilization of lipid reserves of the muscle. Two aspects of this question were considered; the ability of fish muscle lipase to hydrolyze triglycerides containing long-chain fatty acids, and the characteristics of this lipolytic activity compared with those involved in mobilizing depot fat in mammals.

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[1] The fifth paper in the series on utilization of lipids by fish.

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MATERIALS AND METHODS

FISH

Rainbow trout (*Salmo gairdneri*) about 2 years old, weighing 200–300 g, were obtained from a commercial hatchery. They were fed with New Age Complete Trout Fingerling Pellets (J. R. Clark Co. Ltd., Salt Lake City, Utah). The fish used had not been fed for about 20 hr prior to being killed. The fish were stunned, killed by decapitation, and tissue slices were prepared from the anterior portion of the lateral line muscle (Bilinski, 1963). They were cut with scissors into small pieces (approximately 5–10 mm^2), mixed well, and used immediately. The tissue obtained from one fish (3–4 g) was usually sufficient for about 10 incubations.

PREPARATION OF SUBSTRATES

Glyceryl tri(oleate-1-^{14}C) and glyceryl tri(stearate-1-^{14}C) were obtained from the Radiochemical Centre, Amersham, England, and glyceryl tri(palmitate-1-^{14}C) from New England Nuclear Corp., Boston, Massachusetts. Before use, the radioactive triglycerides were purified on silicic acid by the procedure of Borgström (1953) to remove any possible contamination by mono- or diglycerides. For use as substrate, the ^{14}C-triglycerides, diluted with appropriate amounts of nonradioactive triglycerides, were emulsified in buffer solution with fish muscle phospholipid, usually in a ratio of 1 mg phospholipid per 50 mμmoles triglyceride. For emulsification, triglycerides dissolved in benzene were blended in a test tube with a benzene solution of phospholipid. After reducing the total volume to less than 0.3 ml, under a stream of nitrogen, buffer was added and the mixture was blended under a stream of nitrogen by use of a Vortex-mixer until, judging by smell, all benzene had evaporated. The emulsion formed was used directly after preparation.

The phospholipid used for the emulsification of triglycerides was prepared in the following manner: a total lipid extract was prepared from rainbow trout lateral line muscle by the method of Bligh and Dyer (1959) and neutral lipids were removed by elution with chloroform from silicic acid, followed by elution of phospholipids with methanol (Borgström, 1952a).

DETERMINATION OF LIPOLYTIC ACTIVITY

The lipolytic activity was measured by the release of ^{14}C-fatty acids from labelled triglycerides. Incubations were carried out at 35 C, with 350-mg tissue slices, in a total volume of 4.0 ml, and in a constant temperature bath provided with a shaking device. Other incubation conditions were as stated in the tables and figures. At the end of the incubation, the reaction was stopped by adding 20 ml of the extraction mixture described by Carter (1967), in which *n*-heptane was substituted for iso-octane. After homogenization for 2 min in an Omnimixer, the extraction was carried out further, essentially as described by Carter. Free fatty acids were isolated from the heptane phase by the alcoholic NaOH partition method of Borgström (1952b). Radioactivity was determined using the scintillation mixture described by Bjornstad (1966) and internal standards for quenching correction.

THIN-LAYER CHROMATOGRAPHY

Eastman Chromatogram Silica Gel Sheets, type K301R2, were used with a solvent system composed of hexane–diethyl ether–glacial acetic acid, 70:30:1, v/v (Strand et al., 1964). For the determination of the distribution of radioactivity, the portion of the plate containing nonradioactive standards was cut and the compounds were visualized with rhodamine 6G, 0.01% in H_2O. In the other portion of the plate, the silica gel corresponding to the various zones of standard compounds was scraped, extracted with benzene, and radioactivity was measured in the extract by scintillation counting.

RESULTS

In preliminary experiments, various procedures for emulsification of long-chain triglycerides were tested to demonstrate the hydrolysis of tripalmitin by tissue slices from rainbow trout lateral line muscle. These procedures included the use of gum arabic, polyvinyl alcohol, methocel, organic solvents, and sonication, but no significant hydrolysis of tripalmitin occurred in the presence of trout muscle preparations. When, tripalmitin was dispersed with the whole phospholipid prepared from trout lateral line muscle, however, a comparatively high and consistently reproducible hydrolysis took place (Table 1). The phospholipid showed its maximal effect at low concentration and, when higher amounts of phospholipids were used, some decrease in lipolytic activity was observed. The sonication of the triglyceride-phospholipid mixture produced no increase in lipolytic activity. The effect of tripalmitin concentration on lipolysis was investigated with a constant amount of phospholipid (Table 1). Under the conditions used, the maximum rate of hydrolysis took place in 1×10^{-4} M solution.

TABLE 1. Effect of tripalmitin and phospholipid concentration on lipolytic activity.

Tripalmitin M	Phospholipid[a] (*mg/ml*)	Palmitic acid released (*mμmoles/hr*[b])
1×10^{-5}	–	Nil
1×10^{-5}	0.2	4.40, 5.00
1×10^{-5}	1.0	4.41, 4.50
1×10^{-5}	2.0	2.32, 2.62
2.5×10^{-7}	0.2	0.26, 0.26
1.25×10^{-6}	0.2	1.08, 1.30
1×10^{-5}	0.2	4.50, 4.76
1×10^{-4}	0.2	16.30, 17.00
1×10^{-3}	0.2	7.40, 13.20

[a] Phospholipid used to emulsify tripalmitin, according to the procedure described under Materials and Methods.

[b] Incubation conditions: NaK phosphate, *p*H 7.0, 100 mM; 60 min incubation.

In initial experiments, variations were observed in lipolytic activity of tissue slices prepared from the same fish. These variations were found to be due to a nonuniform distribution of lipase along the lateral line muscle, the anterior portion showing higher activity than the posterior (Table 2). This confirms the observation made earlier by Wood (1959b) on uneven distribution of lipase in lingcod fillets. The difficulty was circumvented by using well-mixed fragments of tissue slices taken along the anterior portion of the lateral line muscle. All enzyme assays were conducted at 35 C, since preliminary tests had shown that the activity at this temperature was about three times higher than at 15 C. Heat treatment of tissue slices at *p*H 7.0 and 100 C for 15 min suppressed all the activity.

The effect of *p*H on hydrolysis of tripalmitin was determined between *p*H 6.0 and 8.5 (Fig. 1). This range was covered by use of phosphate and tris buffers. The activity was independent of the nature of the buffer used. Maximal activity was observed at *p*H 7.3. The release of palmitic acid showed a linear

TABLE 2. Distribution of lipolytic activity in lateral line muscle.

Fish no.	Lateral line muscle: Anterior half — Palmitic acid released[a] ($m\mu moles/hr$)	Lateral line muscle: Posterior half — Palmitic acid released[a] ($m\mu moles/hr$)
1	3.48	6.43
2	3.38	8.31
3	3.43	6.30
4	3.24	7.45
Mean 1–4	3.38	7.12

[a] Incubation conditions: 40 mμmoles ^{14}C-tripalmitin; NaK phosphate, *p*H 7.0, 100 mM; 60 min incubation.

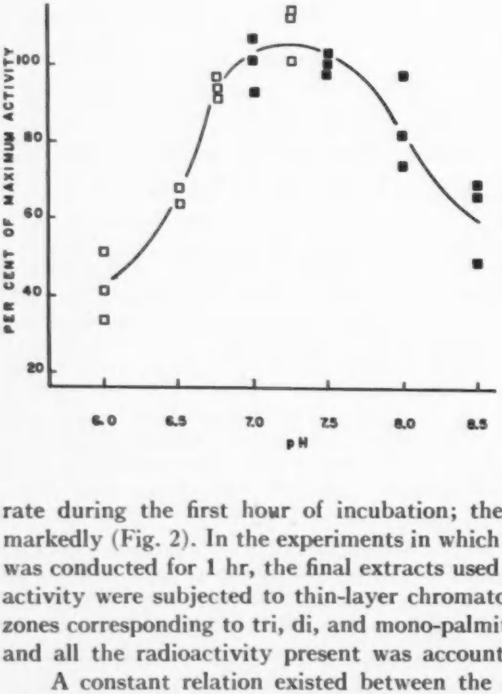

FIG. 1. Effect of *p*H on lipolytic activity. Incubation conditions: 40 mμmoles ^{14}C-tripalmitin; 60 min incubation; □ = NaK phosphate, 100 mM; ■ = Tris, 100 mM.

rate during the first hour of incubation; thereafter, the activity decreased markedly (Fig. 2). In the experiments in which the incubation with tripalmitin was conducted for 1 hr, the final extracts used for the determination of radioactivity were subjected to thin-layer chromatography. It was found that the zones corresponding to tri, di, and mono-palmitin were free from radioactivity and all the radioactivity present was accounted for as palmitic acid.

A constant relation existed between the amount of muscular tissue incubated and the enzyme activity (Fig. 3). The lipolytic activity varied toward different long-chain triglycerides (Fig. 3). The activity toward tripalmitin was more than one order of magnitude greater than that toward triolein. With the sensitive isotopic method used, activity against tristearin was also readily measured, although it was several times smaller than toward triolein.

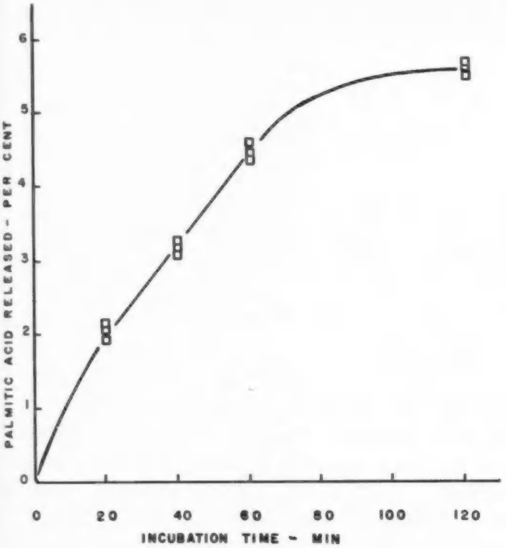

FIG. 2. Effect of incubation time on lipolytic activity. Incubation conditions: 40 mμmoles ^{14}C-tripalmitin; NaK phosphate, *p*H 7.3, 100 mM.

The effects of activators and inhibitors on lipolytic activity were determined at *p*H 7.3 (Table 3). The addition of bovine serum albumin to the incubation medium had a moderate effect on lipolysis. The activity was increased by only approximately 10% at an albumin concentration of between 1 mg/ml and 10 mg/ml and showed no increase at 30 mg/ml concentration. NaF at 25 mM concentration produced approximately 35% inhibition of lipolysis, which was increased to 60% at 200 mM. Protamine caused 80% inhibition at a concentration of 0.3 mg/ml. The -SH group reagent, *p*-chloromercuriphenyl sulfonate, produced approximately 40% inhibition at 0.25 mM concentration.

The effect of epinephrine on lipolytic activity was also investigated. The addition of epinephrine (10 μg/ml) to tissue slices followed by incubation with or without prior homogenization had no effect on lipolysis. It was found, however, that the effect of epinephrine was more apparent when the activity in the intact tissue was first allowed to decline (Table 4). In the procedure used, tissue slices from lateral line muscle were preincubated for 1 hr at 35 C with or without epinephrine (10 μg/ml). The substrate was then added and the incubation was continued for another hour. Under these conditions, the lipolytic activity was about 20% higher in the presence of epinephrine and a statistically significant difference could be established. The effects of cyclic-AMP (1 mM), theophylline (1 mM), and caffeine (1 mM) were tested by using the same procedure, but no conclusive evidence for their effect has been obtained.

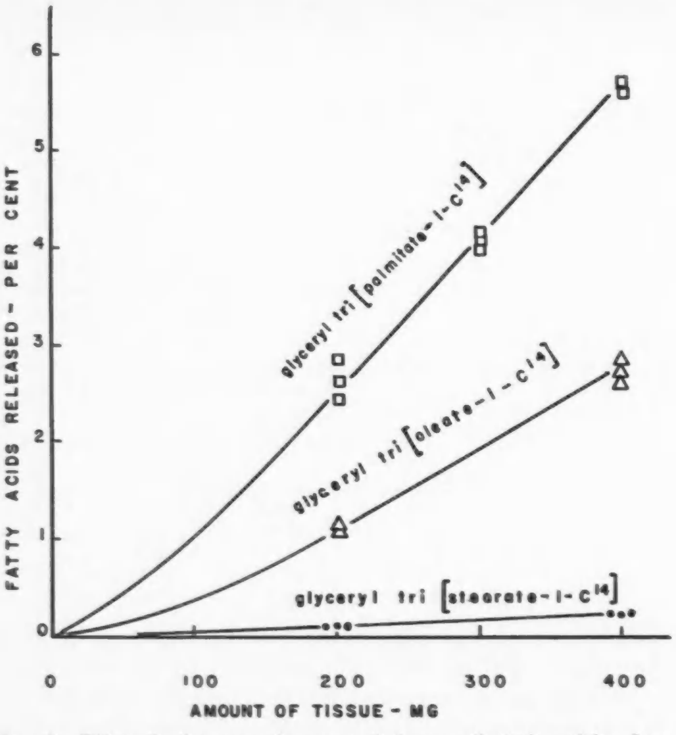

FIG. 3. Effect of substrate and amount of tissue on lipolytic activity. Incubation conditions: 40 mμmoles ^{14}C-triglyceride, 60 min incubation, NaK phosphate, *p*H 7.3, 100 mM.

TABLE 3. Effect of various additives on lipolytic activity.

Compound added	Concentration	Relative activity[a]
Control		100
Bovine serum albumin	1 mg/ml	111, 112
" " "	10 "	109, 112
" " "	30 "	94, 106
NaF	25 mM	66, 68
"	100 "	48, 57
"	200 "	38, 41
Protamine sulfate	0.3 mg/ml	19, 20
p-Chloromercuriphenyl sulfonic acid	0.25 mM	51, 65

[a] Incubation conditions: 40 mμmoles ^{14}C-tripalmitin, 60 min incubation; NaK phosphate, *p*H 7.3, 100 mM — with bovine serum albumin; Tris, *p*H 7.3, 100 mM — with other additives.

TABLE 4. Effect of epinephrine on lipolytic activity.

	Relative activity
Control	100.0±1.42(20)[a]
Control plus epinephrine 10 μg/ml[b]	118.9±2.64(20)
Significance level[c]	$P < 0.001$

[a]Preincubation without substrate in Krebs Ringer phosphate, *p*H 7.3, for 60 min at 35 C, followed by incubation in the same medium with 40 mμmoles ^{14}C-tripalmitin.

[b]Mean value, standard error, and number of determinations.

[c]Student's *t*-test.

DISCUSSION

The results obtained indicate the presence in fish muscle of a lipolytic activity toward long-chain triglycerides that is capable of hydrolyzing depot fat which, in fish, is composed of triglycerides containing predominantly fatty acids with 12–24 carbons. The proper emulsification of substrate is of critical importance to demonstrate this activity in vitro and it was found that the whole phospholipid fraction isolated from fish muscle acts as the proper dispersing agent. The available data suggest a rather specific phospholipid requirement for the inter-reaction between fish muscle lipase and long-chain triglycerides, since various means of emulsification of substrate suitable for the assay of other more active lipases were found unsatisfactory. The demonstration of lipolytic activity against long-chain triglycerides presents difficulties also in the case of mammalian skeletal muscle, and it has been reported that triglycerides containing fatty acids with 12 carbons or more were not hydrolyzed by preparations from rat skeletal muscle (Wallach, 1968). The demonstration of the presence, in trout muscle, of lipolytic activity toward long-chain triglycerides poses a question as to whether this hydrolytic process is different from that promoting cleavage of short-chain triglycerides. The lipolytic activity against long-chain triglycerides in trout muscle, being optimal at *p*H 7.3, differs markedly from the short-chain triglyceride lipase from lingcod muscle, which was found by Wood (1959a) to have optimal activity at *p*H 8.2. These differences show agreement with recent reports on esterolytic and lipolytic activities in mammalian tissues. In mammals, several dissimilarities were observed between the two types of lipolysis; and it was found that, in contrast to the hydrolysis of short-chain triglycerides, in which optimal activity above *p*H 8 was observed, the cleavage of long-chain triglycerides was optimal near neutrality (Schnatz, 1966; Schnatz and Cortner, 1967; Biale et al., 1968). These observations are consistent with the existence of distinctive lipolytic activities, depending on water solubility of triglycerides (Desnuelle and Savary, 1963).

As far as the metabolic process of depletion and deposition of tissue triglycerides is concerned, two types of lipolytic activities are known to occur in mammals: lipoprotein lipase, which plays a role in facilitating the transport of fatty acids from blood triglycerides into the extra-hepatic tissues; and epinephrine-sensitive lipase, which is responsible for hydrolysis of adipose tissue triglycerides during the mobilization of lipid reserves. The two enzymes have not been obtained in pure form and the evidence for their separateness comes from differential effects of various inhibitors and activators, and from a different *p*H optimum (Renold and Cahill, 1965). In relation to the physiological function played by lipase in utilization of depot fat in fish muscle, some properties of trout muscle lipase were compared with those of epinephrine-sensitive lipase from mammalian adipose tissue. Since it was found, with mammals, that the response of lipase to epinephrine is more apparent with tissue slices than tissue extracts (Hollett and Auditore, 1967), the tissue slice preparation was used in our study. The lipolytic activity in trout muscle shows similarities with epinephrine-sensitive lipase by having a *p*H optimum near neutrality and by being inhibited by NaF. It differs, however, from this enzyme by being strongly inhibited by protamine. In mammalian tissue only, the lipolytic activity determined at higher *p*H, which is attributed to lipoprotein lipase, is inhibited effectively by protamine. In studies conducted in vitro with mammalian adipose tissue, it was generally found necessary to add serum albumin to demonstrate maximal rates of lipolysis. The lack of pronounced stimulation of lipolysis by albumin, in the case of trout lipase, could be related to the fact that the incubation system containing muscle preparation is rich in protein material, which may serve as fatty acid acceptor. As far as the action of epinephrine on lipolysis is concerned, it was found that, though epinephrine did not stimulate lipolysis in fresh muscle, it showed some effect by partially preventing loss of activity during preincubation. The implications that this response has for the conditions in vivo is not known. Epinephrine was also found to protect the lipolytic activity from deterioration in preparations from rat adipose tissue (Gorin and Shafrir, 1964; Hollett and Auditore, 1967). In mammals, the response to epinephrine and other lipomobilizing hormones was found to be rather specific for the depot type of adipose tissue (Wenke, 1966), and to vary with different species (Rudman and Di-Girolamo, 1967). In fish, in a study conducted with freshwater species, Farkas (1967) found that the incubation of adipose tissue with norepinephrine did not stimulate the hydrolysis of triglycerides and that the intramuscular injection of norepinephrine did not increase the free fatty acid level in blood. Leibson et al. (1968) found that adrenaline injected intraperitoneally into the lamprey and scorpion fish (*Scorpanea porcus*) raised the concentration of free fatty acid in blood plasma.

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Greater Dispersal of Wild Compared with Hatchery-Reared Juvenile Atlantic Salmon Released in Streams

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When wild juvenile (parr) Atlantic salmon (*Salmo salar* L.) caught by electrofishing, and an equal number of hatchery-reared parr, matched for size with the wild ones, were released at three sites in unfamiliar streams containing resident parr, more hatchery-reared than wild parr could be observed by skin-diving in the areas 1 and 2 weeks later.

Observed mortalities of wild parr were not sufficiently higher than those of hatchery parr to explain this result. Nor could a higher proportion of wild parr be found hiding compared with hatchery parr when one release site was electrofished a week after the release. Wild parr were found in greater numbers at points farther from one of the release sites than were hatchery-reared parr, and also more wild fish passed through a salmon counting fence approximately 100 m from a fourth release site than did hatchery-reared parr.

This greater dispersal of wild parr from the release site compared with hatchery parr has important consequences to estimates of comparative survival between the two stocks.

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INTRODUCTION

COMPARISON OF survival between hatchery-reared and wild trout (*Salvelinus fontinalis* and various species of *Salmo*) which were released together in unfamiliar streams have usually shown the wild fish to be superior (Schuck, 1948; Miller, 1954, 1958; Needham and Slater, 1944; Wales, 1954). The consistency of the result appears to be dependent in part on the presence of a resident population, and on equality of average lengths of hatchery-reared and wild fish. Where no resident population exists, hatchery-reared parr may survive as well as transplanted wild fish (Nielson et al., 1957) and if hatchery-reared fish are on the average larger than the wild fish, the larger hatchery-reared individuals may survive and grow better than wild ones (Mason et al., 1967; Flick and Webster, 1964).

Whether there are behavioural differences which would affect survival is unknown. The measurement of "survival" may itself be biased by as yet unknown differences in behaviour between wild and hatchery-reared fish. The present research was therefore designed to discover whether there might be differences in hiding behaviour and dispersal rates as well as mortality between hatchery-reared and wild salmon parr (*Salmo salar*) when released in populated salmon streams.

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PROCEDURE

A stock of wild salmon parr was obtained by electrofishing a section of a river. These fish were transported to the laboratory where, on the following day, they were measured and individually marked. An equal number of hatchery-reared parr (from wild parents) matched for size with the wild parr (usually to within 2 mm) were also marked. Marking consisted of cold-branding (Everest and Edmundson, 1967) each fish with an individual number and, in addition, most fish were also tagged with modified Swedish smolt tags (Saunders, 1968) or coded polyethylene thread (Saunders and Gee, 1964) both of which were fastened through the dorsal musculature. One, or in a single instance 2, weeks after capture of the wild fish, both stocks were transported to, and released in, a different river from that in which the wild fish were caught (Table 1).

An attempt was made to treat both stocks of fish identically during the holding period in the laboratory. However, wild parr refused to eat the commercially prepared trout-chow provided. The hatchery fish may thus have been planted while in slightly better condition than the wild fish. A few mortalities of both stocks occurred during the holding period which accounted for the slightly different numbers of hatchery and wild parr actually released (Table 1).

The subsequent history of the released fish was followed by skin-diving observations and other methods, depending on the purpose of the experiment. Three different experiments were performed. The treatment of the fish until the time of their release, however, was similar in all three experiments.

In the first experiment, which consisted of four replicates using three sites, the number of released fish which remained within an area approximately 100 m above and below the release site were checked by diving at weekly intervals for 2–5 weeks after planting.

The second experiment, consisting of just one trial, was designed to test for differences in mortality or hiding behaviour after planting. Mortality sometimes resulted from infection around the site of tag attachment on the back of the fish. To test whether tagging caused greater mortality of wild or of hatchery-reared fish, half of the wild and hatchery stocks were left untagged. These untagged fish were identifiable by their brand numbers. Before the fish were released, the release site was partially enclosed with nets 70 m up- and downstream from the release point. Immediately after the diving observations a week later, this partially enclosed area was electrofished to recover fish which might not have been seen because they were hiding.

Finally, the third experiment was designed to test for differences in dispersal rate. This was done by releasing the hatchery-reared and wild fish at two points, one 150 m above, and the other 100 m below a salmon-counting fence. The numbers subsequently passing through the fence were counted.

CAPTURE AND RELEASE SITES

Before fish were released in the first and second experiments an area 20–50 m above and below the release point was electrofished to reduce the number of residents. This provided a habitat at least potentially capable of supporting some of the introduced fish, and at the same time supplied the wild stock which was to be planted the next week at the next site (Table 1).

Three release sites were used in the first experiment and were located on two tributaries to the Northwest Miramichi River, New Brunswick (Fig. 1). The sites were chosen to provide a variety of habitats. One at Little River had a 70-m section below the release site of relatively deep (50 cm) slow-flowing water (approximately 30 cm/sec or less) which was thickly overhung by bushes and trees forming a favorable habitat for brook trout. Above the release site at Little River the terrain was shallower and covered with boulders, and the current was faster and more turbulent. Because the width of this river was

TABLE 1. Information on fish released to compare, in three experiments, sightings, mortality and hiding behaviour, and dispersal.

Place of release	Date of release	Stock origin	No. released	Type of identification mark	Mean length (*cm*)	SD of mean length
Expt. 1. Comparative sightings						
Mile 14	Aug. 1, 1967	Wild, from Little River;	14	Knotted thread	10.78	1.13
		Hatchery	14	" "	10.75	1.67
Square Forks	Aug. 8, 1967	Wild, from Mile 14;	17	" "	8.88	1.43
		Hatchery	19	" "	8.95	1.14
Little River	Aug. 15, 1967	Wild, from Square Forks;	31	Plastic anchored Swedish tag	9.38	1.31
		Hatchery	34	Plastic anchored Swedish tag	9.38	0.92
Mile 14	Aug. 2, 1968	Wild, from Waweig and Little rivers;	30	Knotted thread	11.66	2.16
		Hatchery	31	Knotted thread	11.62	2.16
Expt. 2. Comparative mortality and hiding behaviour						
Little River	Aug. 2, 1968	Wild, from Waweig River;	49	Wire anchored Swedish tag	10.81	1.13
			53	Branded only	10.70	1.04
		Hatchery	53	Wire anchored Swedish tag	10.79	1.36
			51	Branded only	10.66	1.01
Expt. 3. Comparative dispersal						
150 m above Curventon fence, Northwest Miramichi River	July 18, 1968	Wild, from Square Forks;	47	Wire anchored Swedish tag	9.87	1.16
		Hatchery	47	Wire anchored Swedish tag	9.88	1.05
100 m below Curventon fence	July 18, 1968	Wild, from Square Forks;	47	Wire anchored Swedish tag	10.12	1.31
		Hatchery	47	Wire anchored Swedish tag	10.17	1.21

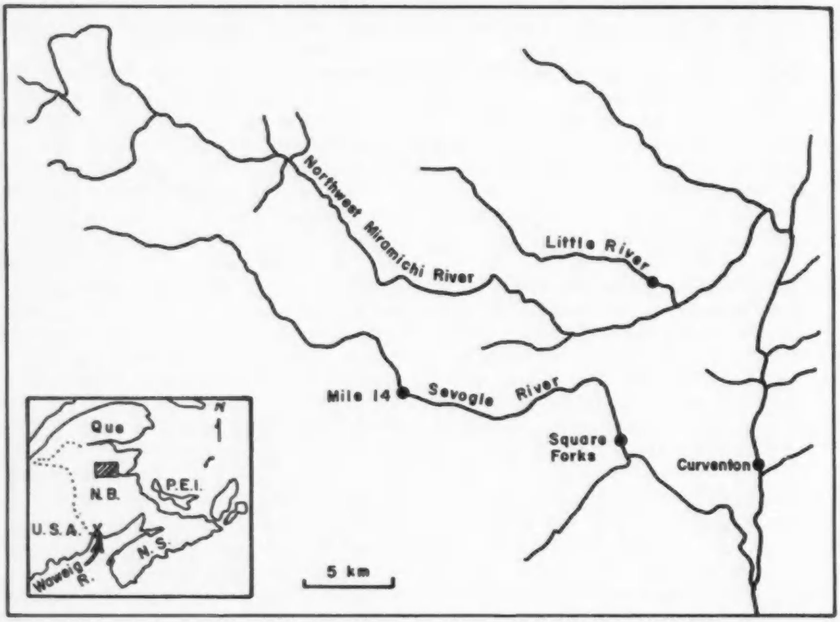

FIG. 1. Release sites of wild and hatchery-reared salmon parr on the Northwest Miramichi and its tributaries. The inset shows the location of the Miramichi River in New Brunswick, and the location of the Waweig River in which some wild fish used in the study were captured.

only about 10 m, it was also used in the second experiment where nets partially enclosed the area 70 m above and below the point of release.

The second and third sites used in the first experiment were located on the Sevogle River which was a much larger tributary than Little River. The two sites, Mile 14 and Square Forks were separated by about 15 km. At both sites the river was about 30 m wide, but at Mile 14 a series of pools formed the main channel whereas at Square Forks the current was rapid (50–60 cm/sec) and the terrain strewn with boulders. There were few trout at either of these sites. Two releases were made at Mile 14, one in 1967, and the second a year later.

Resident salmon parr were plentiful at all three sites, underwater estimates being approximately 2.8, 0.9, and 1.0 parr per 10 m² at Square Forks, Mile 14, and Little River respectively. These figures were probably conservative since more fish could usually be captured by electrofishing the area than were seen when diving.

The fourth release site at the Curventon salmon-counting fence on the Northwest Miramichi River was used only in the third experiment on dispersal rates. The River at the fence was about 100 m wide, the water, 20–30 cm deep, flowed at approximately 40 cm/sec. No diving observations were made or electrofishing done at this site, but from other studies (P. F. Elson, personal communication) the resident population was known to be low (about 0.1/10 m^2).

To supplement stocks of wild fish, some salmon parr were captured by electrofishing in the Waweig River (Fig. 1). Hatchery-reared parr were obtained from Fish Culture Stations at Saint John and Florenceville, N.B., both operated by the Federal Department of Fisheries of Canada.

RESULTS OF THE COMPARISON OF SIGHTINGS OF HATCHERY-REARED AND WILD PARR

Results of the four releases made in the first experiment were consistent, and the data were therefore lumped for statistical analysis. The following percentages of released fish were sighted while diving:

	Wild fish	Hatchery fish
% sighted 1 or more weeks after release	5	15
Total released	92	98

The greater proportion of hatchery than wild parr sighted was statistically significant (chi-square = 4.52, $P < 0.05$).

The trend to observe more hatchery-reared than wild parr 1 week after planting was observed also in experiment 2 at Little River:

	Wild fish	Hatchery fish
% sighted 1 week after release	12	20
Total released	102	104

However, the difference in proportions sighted here was not significant (chi-square = 2.30, $.10 < P < .20$). When the fish recaptured by electrofishing after diving in this experiment were added to those sighted, the trend to find more hatchery than wild parr became highly significant (chi-square = 13.58, $P < .001$):

	Wild fish	Hatchery fish
% sighted, or caught, or both	23	46

These data and those from the first experiment indicated that wild parr may have suffered higher mortality than hatchery parr, or hid more effectively, or dispersed farther from the release site. All three alternatives were examined.

RESULTS OF COMPARING MORTALITY AND HIDING BEHAVIOUR OF WILD AND HATCHERY PARR

Some evidence indicated that wild fish were more susceptible to mortality after handling and tagging than were the hatchery fish:

	Wild fish	Hatchery fish
% dying from fungal infection around the tag before or after planting	3	1
Total tagged	303	306

The difference was significant at the .10 level (chi-square = 3.8, $.05 < P < .10$; the total number of tagged fish included some not shown in Table 1 which were from replicates of experiment 3 where there were no recaptures).

Although this difference was not great, the possibility existed that mortality of wild fish from tagging was sufficiently higher than that of hatchery fish to explain why more hatchery than wild fish were sighted while diving. If tagging caused greater mortality of wild fish than of hatchery fish, then in the second experiment the ratio of tagged to untagged (but branded) wild parr sighted, or captured, or both, should have been smaller than the ratio of tagged to untagged hatchery parr. This was not the case:

	Wild fish	Hatchery fish
Proportion tagged to untagged parr sighted, or captured, or both	13/10 = 1.3	20/28 = 0.7

Certainly in the second experiment then, mortality from tagging appeared unlikely to have caused the greater recovery of hatchery compared with wild fish. In view of this result and the small difference in observed mortalities in the other experiments, there must have been another reason for sighting more hatchery than wild fish. A difference in hiding behaviour was the next possibility examined.

Hidden fish which were missed by the diver could be recovered by electrofishing. This was shown by comparing the total number sighted in the second experiment (33) to the total number captured by electrofishing (64). If wild fish were hiding more effectively than hatchery-reared fish, the proportion of wild fish sighted compared with those captured should have been smaller than for hatchery-reared parr. Again this was not so:

	Wild fish	Hatchery fish
Proportion of parr seen while diving/captured by electrofishing	12/19 = 0.6	21/45 = 0.5

Six individuals, four of them wild parr, were seen that were not captured.

Any difference in hiding behaviour between wild and hatchery-reared parr was apparently insufficient to have caused the diver to sight proportionately more hatchery than wild fish. This conclusion was further supported by observation of the reaction of parr to the diver. The diver could approach to within arm's length of most wild fish, which typically sank to the bottom or edged close to a rock and lay motionless when the diver approached. Hatchery parr, however, were sometimes observed swimming rapidly off when the diver first caught sight of them, and some probably swam off before they were sighted. Therefore, though the wild fish appeared to hide more than hatchery fish in response to the diver, the evasive behaviour of the hatchery parr seemed to be a more effective means of avoiding being seen. The higher proportion of hatchery fish sighted in the first and second experiments apparently was not a result of mortality or hiding behaviour. The only remaining alternative was that hatchery fish tended to remain near the release site whereas wild fish emigrated.

RESULTS OF COMPARING DISPERSAL OF WILD AND HATCHERY PARR FROM THE RELEASE SITE

More of the wild than of the hatchery parr released above and below the Curventon fence were caught in the traps at the fence:

	Wild fish	Hatchery fish
% caught	12[a]	3
Total released	94	94

[a] Three were dead by the time they were discovered. The difference was statistically significant (chi-square = 4.58, $P < 0.05$). The wild fish also appeared to keep moving for a longer time than did hatchery parr. All three hatchery parr recovered entered the traps within 2 days of release whereas the median length of time for the 11 wild fish captured was 6 days (average 11 days, $P = 0.10$, Mann-Whitney U-test).

Although these results were expected if wild fish dispersed farther from the point of release than hatchery parr, the data were capable of a different interpretation. If wild parr had for some reason been less able to maintain their position in the current than hatchery-reared parr, then more wild than hatchery parr would have been recovered drifting downstream from above the fence. All the captured fish were taken in the trap for downstream migrants, though six of the wild parr had been released below the fence and passed upstream without being caught. A weakened condition of wild fish was indicated by the death of 3 of the 11 captured. Wild fish may have been weaker than hatchery parr at the time of release because: (*a*) wild fish did not eat while being held in the laboratory; and (*b*) they may have been more susceptible to fungus infection around the tag than hatchery parr. Although mortality from tagging was insufficient to influence results in the unpolluted waters of Little River, the Northwest Miramichi River at Curventon fence was often heavily polluted (Sprague et al., 1965; the condition has changed little since this publication) and this could have aggravated the poor condition of already weakened fish.

Data from the second experiment at Little River, which was not polluted, were re-examined to see how far hatchery and wild fish had dispersed at that site. The area between the nets was divided visually into approximately equal length quarters, two quarters being adjacent and on either side of the point of release with two distant quarters beyond them. To obtain as large a sample as possible, underwater sightings and electrofishing recaptures were combined. If a fish was both seen and captured, and the position of sighting and capture differed, the position of sighting was used because fish appeared less disturbed by a diver than by someone wading, and because diving observations preceded electrofishing, and the fish were therefore less likely to have been chased from their home location when first sighted under water. Fish were distributed among the distant and near quarters in the following manner:

	Wild fish	Hatchery fish
Distant quarters	15	16
Near quarters	8	31
Totals	23	47[a]

[a] One less than in previous tables because the recapture location of one fish was unknown.

The occurrence of a larger proportion of wild fish in distant quarters was significant (chi-square = 4.32, $P < 0.05$), but the possibility still remained that wild fish might have drifted downstream into the lowermost distant quarter. The following data showed this was not the case:

	Wild fish	Hatchery fish
% of parr seen, or recaptured, or both, downstream from release point	35	40
Total seen, or caught, or both	23	47

The greater dispersal of wild compared with hatchery parr apparently occurred whether the water was polluted or not. Inability of wild fish to maintain their position against the current may have contributed to their dispersal downstream in polluted water, but it was apparently not the sole cause of the different dispersal rates of the wild and hatchery-reared fish.

AGGRESSION AS THE CAUSE OF DISPERSAL

Aggression has been cited as a major cause of emigration of Atlantic salmon (Symons, 1969) and of coho salmon (Chapman, 1962; Ruggles, 1966) from artificial streams. If aggression causes dispersal, factors which decrease aggression should decrease emigration. Salmon parr and trout are less aggressive in still water than in flowing water (Kalleberg, 1958; Hartman, 1965), and *Salmo gairdneri* (Newman, 1956; Onodera, 1962) and *S. salar* (Lindroth, 1955; LeCren, 1965) are reportedly less aggressive than brook trout (*Salvelinus fontinalis*). Therefore, the planted parr that were seen should have been located in slow-flowing water unless brook trout, which inhabit pools rather than riffles (Keenleyside, 1962; Gibson, 1966), were present.

Planted parr usually were located in slow-flowing water, provided no trout were present. At Mile 14, six of the nine planted parr seen were found 10 m upstream from the release point in a pool, and 2 more were in deep water 20 m downstream from the release site. However, at Little River where the slower, deeper water downstream was populated with brook trout, eight of the nine salmon parr sighted during the first experiment were found upstream from the release site in the faster, shallower water. Brook trout were seen charging, nipping, and chasing some of the planted salmon parr which remained in the pool where they were released in experiment 2 at Little River. Apparently planted parr became resident generally in areas not defended by other fish and emigrated from those areas that were defended.

The greater dispersal of planted wild parr compared with hatchery parr may have been the result of a quantitative difference in aggression. Fenderson et al. (1968) reported that hatchery-reared parr tend to be more aggressive than wild parr. Perhaps for this reason hatchery-reared parr were better able to compete for space among the residents than wild fish.

SIGNIFICANCE OF DEGREE OF DISPERSAL TO ESTIMATES OF COMPARATIVE SURVIVAL BETWEEN HATCHERY-REARED AND WILD SALMON PARR

One important consequence of this work is the implication concerning methods of estimating survival rates of wild and hatchery-reared fish. If the degree of dispersal of wild and hatchery-reared parr were not known, the recapture of more wild than hatchery-reared fish at the counting fence would probably have been interpreted as meaning that the survival of wild parr was better than of hatchery parr. Similarly, angling at a distance from the release site might recapture more wild than hatchery-reared fish, which would also infer better survival of wild than hatchery-reared parr.

There was no evidence in the present work to suggest that wild parr did survive better than hatchery-reared fish; in fact, sightings of fish while diving near the release site would suggest the opposite. Even counts of dead fish might give a biased impression of relative mortalities when no barriers to dispersal are present, because wild fish would be more likely to die in locations too far from the release site to warrant careful search, and would therefore more likely be missed. The difference in dispersal between hatchery-reared and wild stock indicates caution must be exercised in interpretation of recovery and mortality data if incorrect conclusions concerning survival are to be avoided.

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Maturity and Fecundity of Walleyes from the Eastern and Western Basins of Lake Erie[1]

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WOLFERT, DAVID R. 1969. Maturity and fecundity of walleyes from the eastern and western basins of Lake Erie. J. Fish. Res. Bd. Canada 26: 1877–1888.

Of 2427 walleyes (*Stizostedion vitreum vitreum* (Mitchill)) examined from Lake Erie in 1964–66, no females were mature at ages below III and all were mature at age V. All male walleyes from the eastern basin were mature at age II, and those from the western basin at age III. Females from the western basin matured at a shorter length and a younger age than those from the eastern basin. Males from the western basin, on the other hand, matured at a longer length and greater age than those from the eastern basin.

Egg production, which was estimated for 60 walleyes from the eastern basin and 78 from the western basin, ranged from 48,000 to 614,000. The number of eggs increased rapidly with increase in total length, but the relation between fecundity and weight was almost linear. Walleyes from the western basin were much more fecund for any given length, weight, or age than those from the eastern basin. Weight was the most accurate indicator of fecundity. The mean diameter of eggs from 32 ripe walleyes was 1.72 mm. Little relation existed between egg diameter and length or age of fish.

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INTRODUCTION

THE ABUNDANCE OF WALLEYES, *Stizostedion vitreum vitreum* (Mitchill), in western and central Lake Erie has declined sharply in the past decade. The walleye was one of the principal species in the commercial and sport fisheries of these two basins of the lake, but total lakewide commercial production dropped from a record 15.4 million lb in 1956 to about 0.7 million lb in 1962. Since 1962 the annual commercial production has been less than 3 million lb. Unlike the populations in the western and central basins of the lake, the abundance of walleyes in the eastern basin has increased since 1955. The annual production in New York waters was only 4000 lb during 1941–55, but increased to 73,000 lb during 1955–66 (highest catch was 107,000 lb in 1963), without an increase in fishing effort.

The abundance of walleyes in the western basin fluctuated tremendously in 1957–67. The population was characterized by the production of "fair to good" year-classes every 3 years — 1959, 1962, and 1965. The natural history of the walleye was studied by the Bureau of Commercial Fisheries to determine the causes of fluctuations in year-class strength. Sexual maturity and fecundity must be understood to explain such variations.

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Recent studies of maturity and fecundity of walleyes in Lake Erie are lacking. The maturity of Lake Erie walleyes was last described by Deason (1933) and the fecundity was last reported on by Leach (1928). The present report describes the relation between length, weight, and age at maturity, and the number of eggs produced by walleyes in the eastern and western basins of the lake.

MATERIALS AND METHODS

Maturity studies were based on 2427 walleyes collected from the eastern and western basins of Lake Erie in April and May 1964–66 (Table 1). Fish from the eastern basin were taken between Barcelona and Dunkirk, New York, a distance of about 27.4 k (17 miles). Walleyes from the western basin were taken between Bono and Port Clinton, Ohio, a distance of about 32.2 k (20 miles). All walleyes were caught by commercial fishermen in trap nets, in small-mesh gillnets (2.75- to 3.13-inch mesh, stretched measure) or in large-mesh gillnets (4.75- to 5.0-inch mesh).

TABLE 1. Dates, localities, and gear of capture of walleyes collected in the eastern and western basins of Lake Erie, 1964–66.

Date	Basin and port	Gear[a]	No. of fish
	Eastern basin		
1964			
Apr. 21	Barcelona	L	123
22	Dunkirk	L	110
1965			
May 5, 11	Barcelona	L	411
Apr. 18	Dunkirk	L	12
1966			
Apr. 18	Barcelona	L	408
18	Dunkirk	S	150
Subtotal			1214
	Western basin		
1964			
Apr. 2, 21	Bono	T	203
6, 13	Bono	L	101
20	Port Clinton	T	117
9, 10, 16	" "	L	62
4, 30	" "	S	64
1965			
Apr. 21	Bono	T	200
26, 27	Port Clinton	T	79
26, 27, 30	Port Clinton	L	83
1966			
May 2, 4	Bono	T	155
Apr. 18, 19	Port Clinton	T	149
Subtotal			1213
Total			2427

[a] T = trap net; L = large-mesh gillnet; S = small-mesh gillnet.

Age-groups are designated by roman numerals corresponding to the numbers of annuli. Because growth of walleyes does not begin before May in Lake Erie, each fish in the samples was credited with an annulus at the edge of the scale.

Ovaries were collected in April 1966 from 78 ripe walleyes caught in the western basin near Port Clinton, Ohio, and 60 in the eastern basin, off Barcelona, New York (Table 2). The ovaries were wrapped in cheesecloth and preserved in 10% formalin. Other data and materials collected included total lengths (in millimeters), weights (grams), and scale samples.

The dependability of volumetric and gravimetric methods for estimating egg production was tested with ovaries from five walleyes. A sample section was removed from each of the anterior, medial, and posterior portions of the right ovary and the volumes and weights of the samples were measured. Volumes were determined by water displacement in a cylinder graduated to 0.1 ml and weights were measured to the nearest 0.01 g on a triple-beam balance after the excess

TABLE 2. Length distributions of walleyes used for fecundity studies, according to basin, 1966.

Length		No. of fish	
mm	*Inches*	Eastern basin	Western basin
393–405	15.5–15.9	–	1
406–417	16.0–16.4	–	–
418–430	16.5–16.9	–	1
431–443	17.0–17.4	–	2
444–455	17.5–17.9	1	2
456–468	18.0–18.4	3	3
469–481	18.5–18.9	5	5
482–494	19.0–19.4	5	5
495–506	19.5–19.9	5	2
507–519	20.0–20.4	5	5
520–532	20.5–20.9	4	5
533–544	21.0–21.4	5	5
545–557	21.5–21.9	5	5
558–570	22.0–22.4	4	4
571–582	22.5–22.9	4	5
583–595	23.0–23.4	2	5
596–608	23.5–23.9	–	2
609–621	24.0–24.4	–	–
622–633	24.5–24.9	–	–
634–646	25.0–25.4	2	–
647–659	25.5–25.9	3	–
660–671	26.0–26.4	1	3
672–684	26.5–26.9	3	1
685–697	27.0–27.4	1	4
698–709	27.5–27.9	1	1
710–722	28.0–28.4	–	4
723–735	28.5–28.9	1	2
736–748	29.0–29.4	–	2
749–760	29.5–29.9	–	1
761–773	30.0–30.4	–	2
774–786	30.5–30.9	–	–
787–799	31.0–31.4	–	1
Total		60	78

moisture on the ovary was removed. The eggs in each sample were then counted and estimates of the total number were calculated by direct proportion for both methods. The remaining eggs were then counted and the actual numbers were compared with the totals calculated for each section by both techniques.

The gravimetric method was more accurate by far for estimating egg production, and estimates based on the samples from the anterior section of each ovary were the most reliable. The percentage errors for the gravimetric method varied from −1.3 to 2.8; the mean of the absolute values was 0.9%. The extreme percentage errors for the volumetric method were −15.0 and 4.7. Consequently, the total egg production for the remaining fish was calculated by the gravimetric method, on the basis of samples from the anterior portion of the right ovary. Samples ranged from 2.5 to 11.8% (average, 4.6%) of the respective ovary weights.

Eggs from 32 ripe walleyes collected off Port Clinton, Ohio, were used to determine the mean egg diameters.

MATURITY OF WALLEYES

Female walleyes in the western basin matured at a shorter length and a younger age than females in the eastern basin, but males in the western basin matured at a longer length and older age than males in the eastern basin.

Maturity of females — Of 1017 females examined, 351 were from the eastern basin and 666 from the western basin. No females matured before age III and all matured by age V. Females from the eastern basin were about 50 and 92% mature at ages III and IV, respectively (Table 3). In comparison, females from the western basin were about 85% mature at age III and almost 99% mature at age IV. Female walleyes from Lake Erie in 1927–28 matured primarily at ages IV and possibly at V and few fish were mature at age III (Deason, 1933).

TABLE 3. Relations between age and sexual maturity of walleyes from the eastern and western basins of Lake Erie, 1964–66. (All males older than age II and females older than age IV were mature.)

Sex and age-group	Eastern basin		Western basin	
	No. of fish	% mature	No. of fish	% mature
Males				
II	108	100.0	364	96.2
Females				
III	61	49.2	161	86.3
IV	94	92.6	74	98.6

The length advantages of the mature over the immature fish in the present study were as follows:

	Age III	Age IV
Eastern basin	12 mm	21 mm
Western basin	42 mm	54 mm

When the percentages of mature walleyes were plotted by 12-mm intervals of total length (Fig. 1), females from the eastern basin were about 25–38 mm

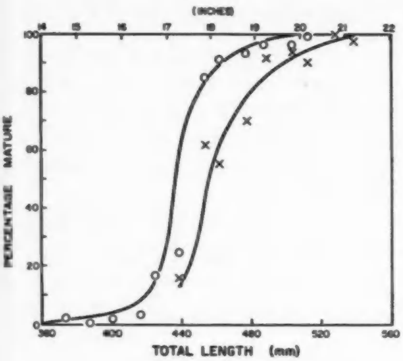

FIG. 1. Relations between maturity and total length in 351 female walleyes from the eastern basin (×) and 666 from the western basin (○) of Lake Erie during the spring, 1964–66. (The curves were drawn by inspection; the curve for the eastern basin females was not completed because of a lack of small specimens in the collections.)

(1 to 1.5 inches) longer at a given percentage maturity than those from the western basin. The total length of the smallest mature female from the eastern basin was 433 mm (17 inches); about 50% were mature at 450 mm (17.75 inches) and all were mature at 540 mm (21 inches). The smallest mature female from the western basin was 378 mm (15 inches) long; 50% were mature at 430 mm (17 inches) and all were mature at 510 mm (20 inches).

Deason (1933) reported that some females longer than 381 mm (15 inches) from Lake Erie were immature. Several large (429–559 mm, 16.9–22.0 inches) immature females were noted in Lake of the Woods by Carlander (1945), who suggested that walleyes might skip spawning seasons or become sterile. No such evidence was found in the present study.

Maturity of males — Of 1410 males examined, 863 were from the eastern basin and 545 from the western basin. All age-II males were mature in the eastern basin but only 96% of those in the western basin were mature (Table 3), even though they averaged 37 mm longer than eastern basin males. Among age-II fish in the western basin, mature males were 24 mm longer than immature males. No age-I males were taken in either basin. In 1927–28, Deason (1933) found that few males matured at age II and maturity was not complete until after the third year of life.

Decrease in age at maturity — The decrease in average age at maturity of walleyes since 1927–28 has accompanied a large increase in the growth rate. First-year calculated lengths of walleyes in 1927–28 averaged 100 mm (Deason, 1933), as compared with about 250 mm in the present study. Hile (1954) also showed that age at maturity decreased with increase in growth rate in Saginaw Bay.

The changes in age and size at maturity and rate of growth coincided with the disappearance of the blue pike (*Stizostedion vitreum glaucum*) and the sauger (*Stizostedion canadense*), and with an extremely marked reduction in the abundance of walleyes (Regier et al., 1969). The walleye presently is the only major terminal predator in Lake Erie and its excellent growth most likely is due to the decrease in interspecific as well as intraspecific competition.

FECUNDITY OF WALLEYES

Fecundity of walleyes from the eastern and western basins of Lake Erie ranged from 48,000 to 614,000. The relation between egg production and total length was curvilinear, whereas the relation between fecundity and weight was almost linear. Walleyes from the western basin were more fecund for any given length, weight, or age than those from the eastern basin. Weight was the most accurate indicator of fecundity.

Relation between fecundity and length — Sixty walleyes from the eastern basin, 452–726 mm long, produced between 54,000 and 465,000 eggs (Fig. 2); 78 from the western basin, 396–793 mm long, produced between 48,000 and 614,000 eggs (Fig. 3). In both basins the relation between number of eggs and length of fish was curvilinear, and the number of eggs produced (Table 4) increased rapidly with an increase in length. A plot of the logarithms of the numbers of eggs and the logarithms of the lengths of fish from the two basins demonstrates the differences in fecundity between the two groups (Fig. 4).

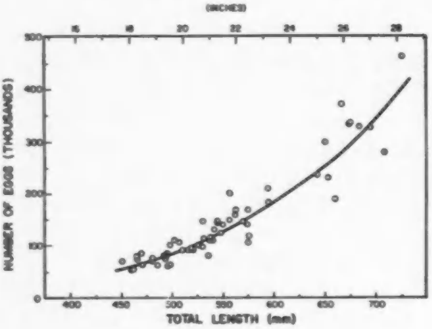

FIG. 2. Relation between number of eggs and total length in 60 walleyes from the eastern basin of Lake Erie in April 1966. (The curve was fitted by the regression of Fig. 4.)

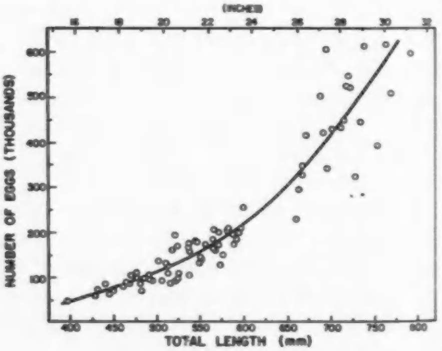

FIG. 3. Relation between number of eggs and length in 78 walleyes from the western basin of Lake Erie in March and April 1966. (The curve was fitted by the regression of Fig. 4.)

TABLE 4. Relations between the average number of eggs and the length of walleyes from the eastern and western basins of Lake Erie.

Midpoint of size group		Eastern basin			Western basin		
Inches	*mm*	No. of fish	Mean egg production	Mean deviation	No. of fish	Mean egg production	Mean deviation
15.5	394	0	–	–	1	48,000	–
16.5	419	0	–	–	1	59,000	–
17.5	445	1	60,000	–	4	73,000	6,592
18.5	470	8	70,000	9,183	8	95,000	9,675
19.5	496	11	80,000	15,577	7	98,000	12,799
20.5	521	8	104,000	13,285	10	128,000	28,922
21.5	546	11	132,000	23,953	10	156,000	19,106
22.5	572	7	143,000	18,714	9	175,000	21,933
23.5	597	2	197,000	13,278	7	204,000	17,890
24.5	623	0	–	–	0	–	–
25.5	648	4	253,000	22,984	0	–	–
26.5	673	5	312,000	49,002	4	345,000	35,009
27.5	699	2	304,000	23,738	5	459,000	74,722
28.5	724	1	465,000	–	6	464,000	64,190
29.5	750	0	–	–	3	482,000	86,629
30.5	775	0	–	–	2	560,000	53,946
31.5	801	0	–	–	1	595,000	–

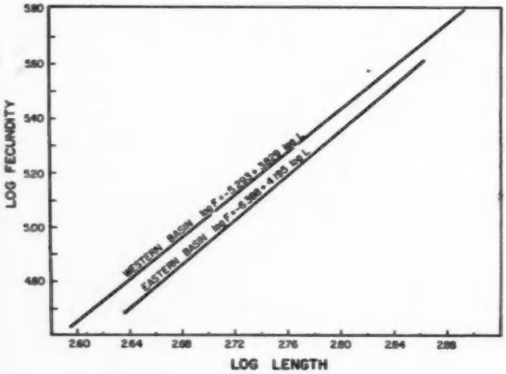

FIG. 4. Relations between number of eggs and length in walleyes collected from the eastern and western basins of Lake Erie in March and April 1966.

The logarithmic forms of the fecundity–length equations are:

$$\text{Eastern basin } \log F = -6.388 + 4.195 \log L,$$
$$\text{Western basin } \log F = -5.293 + 3.829 \log L,$$

where F = fecundity and L = total length in millimeters. These equations were derived as simple least squares fits to the ungrouped data.

A simple analysis of covariance on these data showed that there was homogeneity of variance between the eastern and western basin samples.

The slopes of the two regression lines were then tested (F-test) and found not significantly different at $P = 0.01$. An F-test of the intercepts of the two lines showed a highly significant difference at $P = 0.01$: that is, the average fecundity of western basin walleyes is substantially higher than that of eastern basin walleyes despite similarities in the average lengths of individuals of the two groups.

Langlois (1945) reported that a 27.5-inch (698 mm) walleye from Lake Erie contained 238,110 ova. Carlander (1945) stated that the egg production of nine fish (length range, 343–556 mm) from Lake of the Woods, Minnesota, ranged from 35,000 to 137,000 eggs. Eschmeyer (1950) reported that 50 walleyes, 16.0 to 30.2 inches (407–767 mm) long, from Lake Gogebic, Muskegon River, and Saginaw Bay, Lake Huron, produced between 36,871 and 615,166 eggs.

Relation between fecundity and weight — Plots of the numbers of eggs and weights of fish from the two basins (Fig. 5 and 6) indicated the relation was nearly linear for both basins. Plots of the logarithms of the numbers of eggs and the logarithms of the weights of the fish from the two basins (Fig. 7)

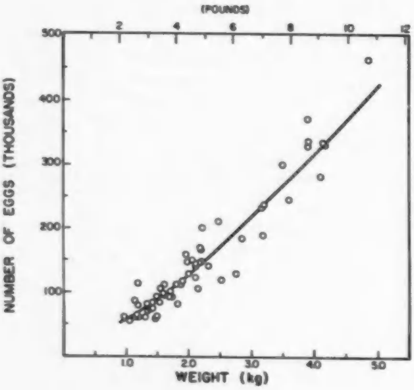

FIG. 5. Relation between number of eggs and weight in 60 walleyes taken from the eastern basin of Lake Erie in April 1966. (The curve was fitted by the regression of Fig. 7.)

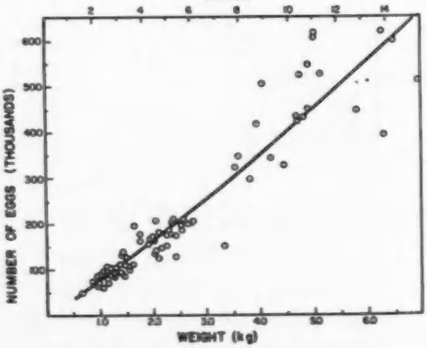

FIG. 6. Relation between number of eggs and weight in 78 walleyes taken from the western basin of Lake Erie in March and April 1966. (The curve was fitted by the regression of Fig. 7.)

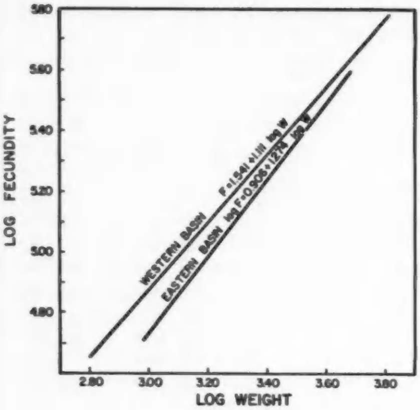

FIG. 7. Relations between number of eggs and weight in walleyes taken from the eastern and western basins of Lake Erie in March and April 1966.

indicated the differences between them. The logarithmic forms of the fecundity–weight equations are:

$$\text{Eastern basin } \log F = 0.906 + 1.274 \log W,$$
$$\text{Western basin } \log F = 1.541 + 1.111 \log W,$$

where F = fecundity and W = weight in grams. These equations were derived as simple least squares fits to the ungrouped data.

A simple analysis of covariance of these data showed homogeneity of variance between the two groups. A comparison of the slopes (F-test) of the two regressions showed that they are not significantly different. An F-test of the intercepts of the two lines showed a highly significant difference. That is, if mean weights of two groups of walleyes (one from the eastern basin and one from the western basin) were the same, average fecundity of the western basin group would be higher.

The average number of eggs per pound of fish was 27,795 (range 18,723–44,052) in the eastern basin and 37,507 (range 25,539–55,895) in the western basin. Leach (1928) stated that walleyes from Lake Erie produced about 45,000 eggs per pound of fish. Estimates of eggs per pound of fish for other lakes were 13,500 for Norris Reservoir (Smith, 1941), 22,700 for Lake of the Woods (Carlander, 1945), and 26,328–30,817 for Lake Gogebic, 29,899–43,616 for the Muskegon River, and 41,680 for Saginaw Bay (Eschmeyer, 1950).

Relation between fecundity and age — The fecundity of walleyes of common age was greater in the western than in the eastern basin (Table 5). The average number of eggs for age-III fish, for example, was 91,000 in the western basin and 63,000 in the eastern basin. As expected, mean egg production increased progressively with age. The most fecund fish was an age-IX female from the western basin that produced nearly 614,000 eggs.

Comparison of adjusted mean fecundity, length, weight, and age — The preceding sections have shown that fecundity is higher in the western basin than in the eastern basin when it is compared with one variable at a time

TABLE 5. Relations between the average number of eggs and the age of walleyes from the eastern and western basins of Lake Erie.

	Eastern basin			Western basin		
Age-group	No. of fish	Mean egg production	Mean deviation	No. of fish	Mean egg production	Mean deviation
III	5	63,000	9,088	14	91,000	20,714
IV	33	99,000	22,230	37	140,000	36,671
V	7	148,000	23,385	5	213,000	17,180
VI	3	255,000	77,875	2	261,000	86,734
VII	5	232,000	32,191	3	374,000	85,286
VIII	6	302,000	30,020	3	364,000	43,446
IX	0	–	–	9	502,000	64,935
X	1	465,000	–	3	503,000	75,430
XI	0	–	–	2	520,000	75,662

(length, weight, and age). The data were also analyzed using a covariance technique which adjusted the mean lengths, weights, and ages of walleyes from each basin to a common value and then compared the differences in adjusted mean fecundity (Snedecor, 1956). The values for the adjusted means by basin and their differences are as follows:

	Length	Weight	Age
Western basin	167,100	167,500	174,500
Eastern basin	133,700	134,600	127,700
Difference	33,400	32,900	46,800

These differences were highly significant and clearly showed that fecundity, even when adjusted for differences in length, weight, and age, was always higher in the western basin.

Indicators of fecundity — In a study of reproductive potential, knowing what measurement is most closely correlated to fecundity is necessary to estimate the total egg deposition of a population. Therefore, a multiple regression analysis of fecundity on length, weight, age, and egg weight was made for each basin to determine the relative importance of these variables as indicators of fecundity.

The standard partial regression coefficients (log base 10) of each variable on fecundity for each basin were as follows:

	Length	Weight	Age	Egg weight
Eastern basin	0.3770	0.4375	0.2169	–0.1518
Western basin	0.1989	0.7847	0.0651	–0.1315

Weight was the most accurate indicator of fecundity in both basins. In the eastern basin weight was closely followed by length, but in the western basin weight was almost four times as accurate an indicator as length. Age and egg weight were both poorly correlated with fecundity. This was expected since walleyes were at different stages of maturity when sampled and egg size varied accordingly.

Egg diameters — No relation was found between egg diameter and length or age of walleyes. The mean diameter of 1600 eggs (50 from each of 32 walleyes, 458–769 mm long) was 1.72 mm (range 1.40–2.04 mm). The mean diameter of walleye eggs found by earlier investigators was somewhat larger: 2.03 mm for Lake Erie (Leach, 1928) and 2.12 mm for an Indiana lake (Miles, 1915).

Possible reasons for differences in fecundity in the eastern and western basins — Scott (1962) found that egg production of rainbow trout (*Salmo gairdneri*) was directly proportional to food supply, and Svärdson (1949) theorized that a selection toward females with an inherent ability to produce larger numbers of eggs may exist under conditions of low intraspecific competition for food and space. If, however, intraspecific competition is high, the selection would be toward females that produce a smaller number of eggs. The available data on population density and intraspecific competition among walleyes in Lake Erie are too scant for judgment of their effects on egg production.

Differences in the length–weight relation of fish from the two basins would be expected to affect fecundity, but a comparison of the relations could not account for the substantial differences in fecundity between the two basins.

A possible explanation for differences in fecundity between the two groups of fish is that separate subpopulations inhabit the basins and the fecundity relationships could have diverged. McGregor (1923) found that some races of chinook salmon (*Oncorhynchus tshawytscha*) could be separated by egg counts. Aro and Broadhead (1950) found differences in the reproductive potential of sockeye salmon (*Oncorhynchus nerka*) spawning in two lakes of the same river system. They concluded that the differences in egg counts of fish of the same size and age were due to the racial characteristics of the fish from each area. I believe that the walleye stocks in the eastern and western basins of Lake Erie are discrete populations and rarely intermix at any stage in life. Of almost 500 recoveries from 4000 walleyes tagged in the western basin (Wolfert, 1963), only two fish were recaptured in the eastern basin. The walleye population along the U.S. shore of the central basin is today almost nonexistent.

ACKNOWLEDGMENTS

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Similarity of Otolith Nuclei in Spring- and Autumn-Spawning Atlantic Herring in the Southern Gulf of St. Lawrence

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MESSIEH, SHOUKRY N. 1969. Similarity of otolith nuclei in spring- and autumn-spawning Atlantic herring in the southern Gulf of St. Lawrence. J. Fish. Res. Bd. Canada 26: 1889–1898.

The optical appearance of otolith nuclei was found inadequate for separating spring and autumn spawning herring in the southern Gulf of St. Lawrence. The nuclei of both groups of spawners in 1966 ranged from distinctly hyaline to completely opaque, and the right and left nuclei differed in some fish. Frequency distributions of hyaline and opaque nuclei and of combinations of these types were similar for the two groups of spawners. Frequency distributions of fish length at time of formation of the first annulus did not differ in relation to type of otolith nucleus though they did differ in relation to the widths of the central areas of the scales. The similar frequencies of the various types of nuclei in the two spawning groups may result from the variable hydrographic conditions in the area.

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INTRODUCTION

EUROPEAN INVESTIGATORS have generally agreed that spring- or summer-spawned Atlantic herring (*Clupea·harengus* L.) in the eastern North Atlantic can be separated from autumn- or winter-spawned fish on the basis of optical appearance of the otolith nuclei. Birtwistle and Lewis (1922) found that Irish Sea herring spawned in autumn or winter had otoliths with hyaline nuclei, and those spawned in spring or summer had otoliths with opaque nuclei. Einarsson (1951) separated summer–autumn and winter–spring spawning stocks off Iceland on the same basis. Parrish and Sharman (1958) found the difference in appearance satisfactory for separating most summer or autumn spawners from winter or spring spawners in North Sea herring. Nuclei were assumed to differ because the otolith of a herring hatched in late summer or autumn is formed mostly in "winter" conditions, whereas those of herring hatched in winter or spring are formed and grow mostly in "summer" conditions.

The European practice encouraged several workers to apply the principle in studying western North Atlantic herring (Graham, 1962; Tibbo and Graham, 1963; Humphreys, 1966; and Hourston, 1968). But Hourston, examining herring from Newfoundland waters, found otolith nuclei ranging in a virtually unbroken series from distinctly "spring-spawned" to distinctly "fall-spawned" types. Humphreys made similar observations on fish from the same area. Both deduced that the herring spawn over almost the entire year.

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The study reported in this paper was undertaken to determine whether the nuclei are useful in separating spring and autumn spawners in the southern Gulf of St. Lawrence. Frequency distributions of the types of nuclei were determined in spring and autumn spawners of 1966, and the distributions in the spring spawners in relation to back-calculated lengths at the end of the 1st year's growth. Back-calculated lengths have been widely used to determine the season during which herring stocks were spawned (Cushing, 1968; Wood and Foster, 1966; and Zijlstra, 1958). Also, distributions of the types of nuclei in spring spawners were compared with those of scales with narrow and wide central areas. Jean (1956) concluded, on the basis of the ratio of the 1st year's growth of the scales to that of the second, that spring-spawning fish in the southern Gulf of St. Lawrence may have hatched in either spring or autumn.

MATERIALS AND METHODS

Otoliths and scales were taken from 1050 spring-spawning fish caught in six widely separated areas in the southern Gulf of St. Lawrence during May, and otoliths from 400 autumn-spawning fish caught in three of the areas during August. Total lengths were recorded for all fish. Ages were estimated from both otoliths and scales.

Otoliths were immersed in 70% alcohol and examined against a black background under a 10× binocular microscope, with reflected light. As the otolith nuclei of both groups of spawners ranged from distinctly hyaline to completely opaque and even the right and left nuclei of the same fish sometimes differed (Fig. 1), the nuclei were grouped on the basis of four types: hyaline (dark against black background), opaque (white), intermediate (partly hyaline, or between hyaline and opaque), otolith pair different (representing any two of these three types).

Scales were examined under a 20× microprojector and reflected images were measured for calculating the ratios of growth in the 1st year to that in the first two, as indexes of widths of the central areas and for calculating fish length at time of formation of the first annulus according to Lee's (1920) method. The following formulae were used:

$$l_1 = (\mathrm{L}-a)\cdot\frac{v_1}{\mathrm{V}} + a$$

and

$$r = v_1/v_2$$

where l_1 = fish length estimated at time of first annulus formation; L = fish length at time of capture; a = fish length at time of scale formation; v_1 = scale radius to first annulus; V = scale radius to anterior margin; r = scale ratio; v_2 = scale radius to 2nd annulus.

The length a was estimated by extrapolating the regression line for the relation between L and V for normal scales, and was calculated as 40 mm. This value is nearly equal to the fish length at metamorphosis (Jean, 1956). Fish with abnormal or regenerated scales and those showing different age estimates by scale and otolith methods were excluded from the calculations. This reduced the data for analysis of back-calculated lengths to 990 fish.

Scale-ratio frequency distributions were separated into theoretical normal curve components (scales with narrow and wide central areas) by plotting cumulative percentage frequency distributions of the scale ratios on probability graph paper, a procedure described by Harding (1949) and discussed by Cassie (1950, 1954). The normally distributed groups so determined were then replotted as single frequency distributions with the ordinate (scale ratio) corresponding to 50% and hence representing the mean of the group, and the range between 15.9 and 84.1% representing two standard deviations.

The χ^2 test for a (2 × N), where N was the number of length groups, was carried out to examine the differences in l_1 distributions between fish with hyaline and opaque nuclei.

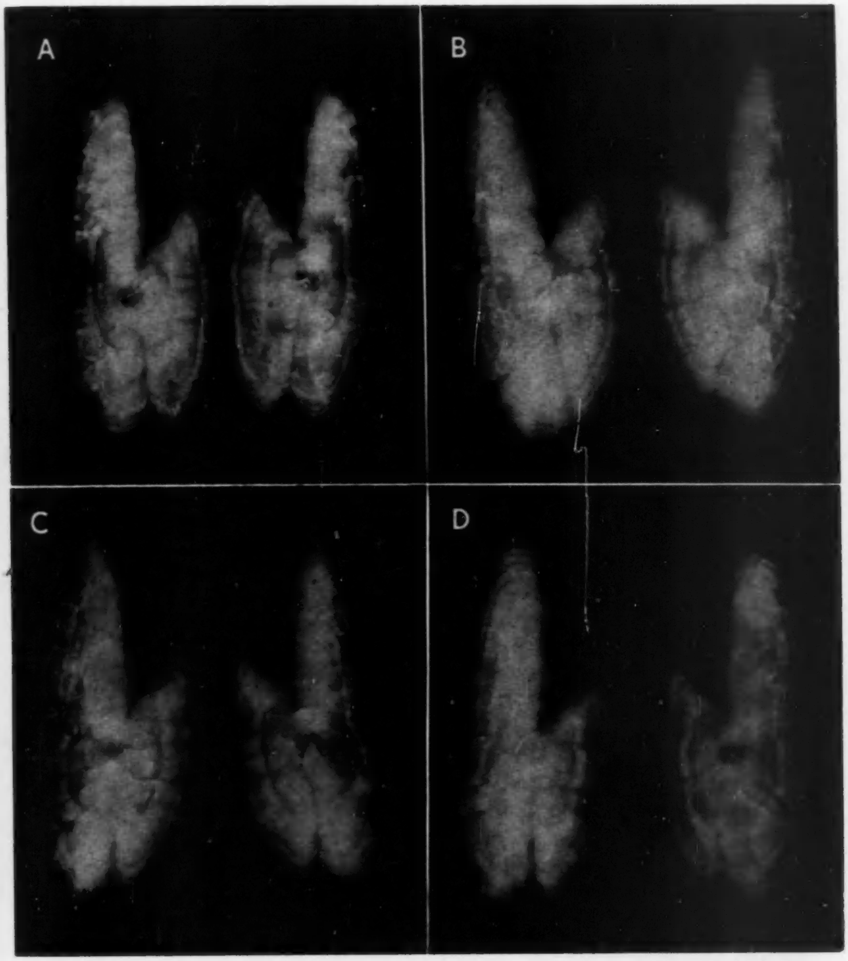

FIG. 1. Photomacrographs of Atlantic herring otoliths from the southern Gulf of St. Lawrence showing four types of nuclei: A, hyaline; B, opaque; C, intermediate; D, pair different.

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To find the source of variation in appearance of otolith nuclei within and between year-classes, an analysis of variance of l_1's was carried out on a two way table (i.e., otolith × year-class).

RESULTS

The four types of nuclei were represented in the two groups of spawners in similar proportions, though the percentage of hyaline nuclei tended to be higher in spring (43%) than in autumn (36%) spawners (Fig. 2).

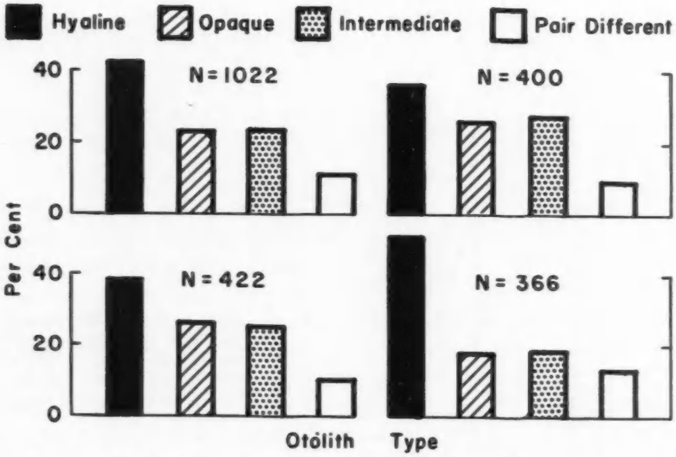

FIG. 2. Frequency distributions of types of nuclei in spring-spawning (*upper left*) and autumn-spawning (*upper right*) herring, and in herring with narrow central areas on the scales (*lower left*) and those with wide areas (*lower right*).

DISTRIBUTIONS OF OTOLITH TYPES IN RELATION TO WIDTH OF CENTRAL AREA OF SCALES

Scales of spring spawners showed a wide range in width of central area, the ratios of the growth in the 1st year to that in the first 2 years ranging from .25 to .80. Jean (1956), from similar observations, suggested that the spawning population may be a composite of fish hatched at different seasons.

For the cumulative percentage frequencies of the ratios of scale growth in the 1st year to that in the first 2 years, a sigmoid curve with one point of inflection provided a good fit for both observed and calculated data (Fig. 3). This indicated two normal curves, possibly representing spring- and autumn-spawned fish. The initial and final points were excluded from the figure as they indicated two minor distributions, possibly representing fish hatched early in the spring or late in the fall (Jean, 1956). However, these points represented only 3% of the total observations.

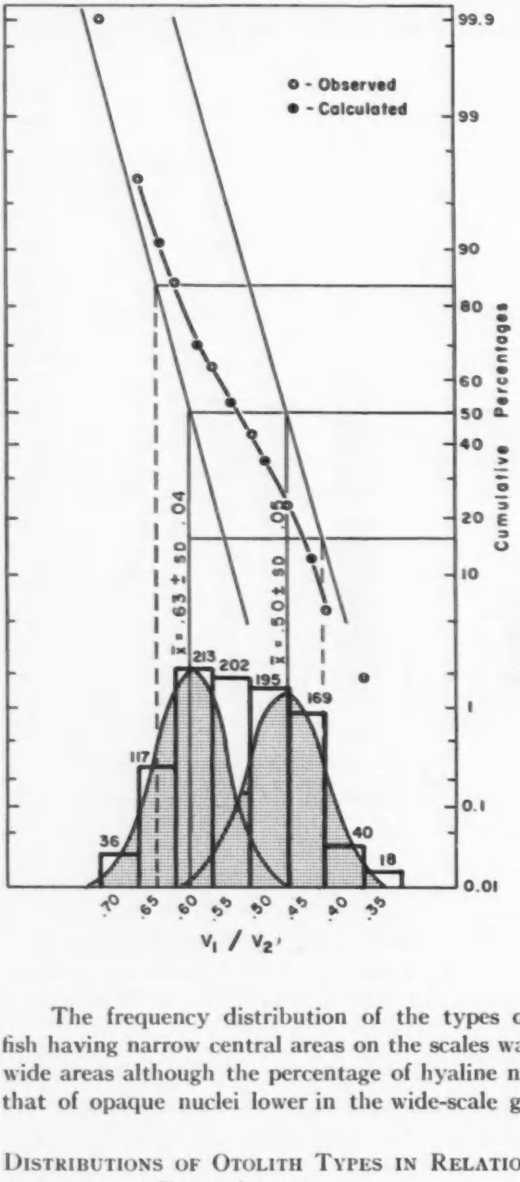

FIG. 3. Probability graph showing probable frequency distributions of scales with narrow and wide central areas. (V_1/V_2: ratio of scale growth in 1st year to that in first two).

The frequency distribution of the types of otolith nuclei (Fig. 2) for fish having narrow central areas on the scales was similar to that for fish with wide areas although the percentage of hyaline nuclei tended to be higher and that of opaque nuclei lower in the wide-scale group.

DISTRIBUTIONS OF OTOLITH TYPES IN RELATION TO FISH LENGTH AT FORMATION OF FIRST ANNULUS

The l_1's back-calculated for 990 spring-spawning fish ranged from 80 to 180 mm, total length (Fig. 4, *left*). As expected, the distributions of l_1 for

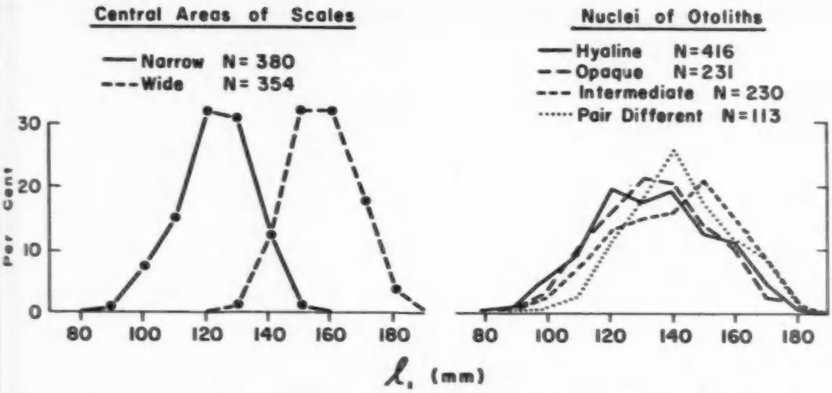

FIG. 4. Frequency distributions of fish lengths at formation of first annulus (l_1's) in relation to width of central areas of scales (*left*) and the various types of otolith nuclei (*right*).

the two scale groups overlapped little. Both were unimodal and approximately normal but the ranges were different. In contrast, l_1's for the four otolith types (Fig. 4, *right*) all had similar ranges and means.

In all year-classes from 1958 to 1963 (Fig. 5) the l_1's for fish with wide and narrow central areas on the scales overlapped little and their means (Table 1) were significantly different, whereas l_1's for hyaline and opaque otolith nuclei (Fig. 5) were roughly congruent and their means were similar (Table 2). The χ^2 test showed no significant difference in l_1 distributions between hyaline and opaque nuclei in any of the year-classes (Fig. 5). Distributions for the other two types of nuclei (not shown in Fig. 5) were similar.

The differences in mean l_1's between the various otolith types within year-classes were not significant ($F = 3.26$; $F_{.05(3,15)} = 3.29$) whereas the differences between the year-classes were highly significant ($F = 17.16$; $F_{.05(5,15)} = 2.90$).

DISCUSSION

This study showed that differences in optical appearance of otolith nuclei should not be taken as a criterion for separating the spawning groups of herring in the southern Gulf of St. Lawrence.

The similar distributions of the four types of nuclei in the spring and autumn spawners (Fig. 2) and the assumption that winter conditions produce hyaline nuclei and summer conditions opaque nuclei suggest that the young of the two groups develop under a great variety of conditions rather than under two distinctly different sets of conditions, each set being more or less uniform within itself.

The consistent presence of all otolith types in both spring- and autumn-spawning fish cannot be explained by the Hourston (1968) – Humphreys

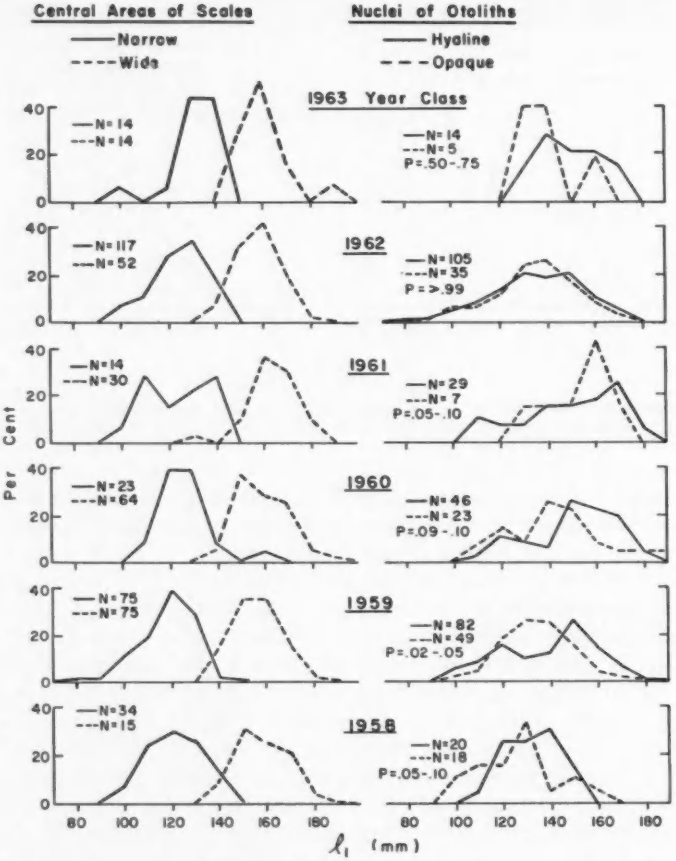

FIG. 5. Frequency distributions of l_1's in relation to widths of central areas of scales and of two types of otolith nuclei for six year-classes of herring. N: number of fish; P: probability corresponding to χ^2 values.

TABLE 1. Mean l_1's (mm) of six year-classes of herring in relation to widths of central areas of scales.

	Narrow area		Wide area	
Year-class	No. of fish	$\bar{l}_1$	No. of fish	$\bar{l}_1$
1958	34	123.5±10.91[a]	15	153.0±12.22
1959	75	120.5±11.26	75	157.9± 9.01
1960	23	129.8±10.39	64	161.1±10.86
1961	14	126.4±14.20	30	167.2±10.56
1962	117	127.1±12.11	52	160.4± 8.28
1963	14	133.6±10.27	14	164.6±11.00

[a] One standard deviation of the sample.

TABLE 2. Mean l_1's (mm) for six year-classes in relation to type of otolith nuclei.

	Hyaline		Opaque		Intermediate		Pair different	
Year-class	*n*	$\bar{l}_1$	*n*	$\bar{l}_1$	*n*	$\bar{l}_1$	*n*	$\bar{l}_1$
1958	20	132.5±11.64[a]	18	126.1±16.85	16	128.8±16.68	12	130.0±20.00
1959	82	139.9±21.17	49	134.1±14.42	48	134.2±19.22	24	144.2±17.67
1960	46	150.9±17.87	23	143.0±20.55	18	144.4±16.17	11	155.4±16.35
1961	29	149.7±21.46	7	152.9±13.80	13	146.5±24.68	3	143.3±23.09
1962	106	136.9±19.62	35	135.7±16.68	42	132.4±22.18	29	136.2±19.16
1963	14	149.3±13.28	5	140.0±12.25	7	138.6±21.93	6	141.7±11.69

[a] One standard deviation of the sample.

(1966) deduction that the herring spawn over almost the entire year. Jean (1956) showed that in this area spawning is limited to spring and autumn.

Jean's (1956) conclusion from his study on Bay of Chaleur herring that spring-spawning fish hatched either in the spring or in the autumn could explain the presence (Fig. 2) of the various otolith types in the two spawning groups in the same frequency pattern. But it cannot explain the similarity in l_1 distributions of fish of the various otolith types (Fig. 4) or in distributions of the otolith types in relation to widths of the central areas of the scales (Fig. 2). Therefore another explanation has to be sought.

Hydrographic investigations in the study area (Lauzier, 1957; Lauzier et al., 1957) showed that the waters are highly stratified during the summer, a warm surface layer of lower salinity being separated from the cold layer by a sharp thermocline (Fig. 6). Decreasing salinities are generally associated with land drainage, which is greatest in the spring. As the salinities decrease, the surface layer becomes thinner and the gradient in the thermocline becomes steeper. Vertical mixing is confined to an ever-thinning surface layer as spring

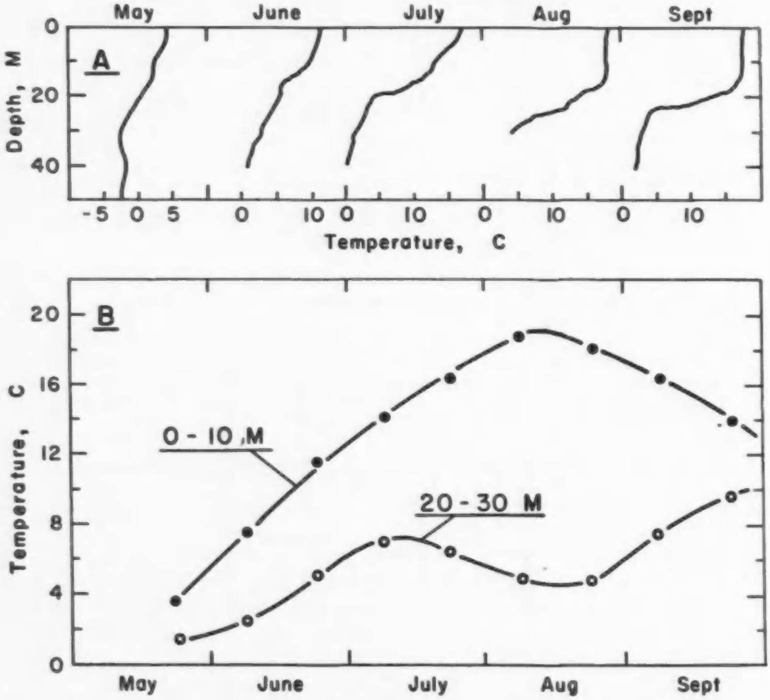

FIG. 6. A, Monthly progress in development of surface layer in southern Gulf of St. Lawrence (adapted from Lauzier et al., 1957). B, Semi-monthly averages of water temperatures at two depths from North Rustico, P.E.I. (adapted from Lauzier, 1957).

progresses to summer. The year-to-year variations of surface salinities are correlated with the variations in the run-off of the St. Lawrence River. The run-off, and hence salinity, undergoes a very wide range of variations; and increasing temperatures are associated with decreasing salinities and vice versa.

Thus herring larvae transported in different directions from their spawning grounds are likely to encounter different and variable hydrographic conditions during and after the period of otolith nucleus formation. This is presumably the reason for the great variation observed in nuclei of otoliths of Gulf of St. Lawrence herring of the same year-class and the same spawning groups.

A similar situation was described for Dunmore herring in Irish waters. Wood and Foster (1966) found that winter spawners, though constituting a self-contained stock, had a wide range of otolith types and that these were not associated with any particular part of the spawning period.

As early growth would be expected to differ appreciably between year-classes with such variable hydrographic conditions on the rearing grounds, the differences between year-classes provide further support for this hypothesis.

ACKNOWLEDGMENTS

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Decapod Crustacea of the Beaufort Sea and Arctic Waters Eastward to Cambridge Bay, 1960–65

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SQUIRES, H. J. 1969. Decapod Crustacea of the Beaufort Sea and arctic waters eastward to Cambridge Bay, 1960–65. J. Fish. Res. Bd. Canada 26: 1899–1918.

The CGS *Salvelinus* collections of decapod crustaceans from south Beaufort Sea to Cambridge Bay in 1960–65 comprised 1830 specimens of 15 species. Five of the species were not previously recorded from this area. Most abundant species were *Sabinea septemcarinata* and *Eualus gaimardi. Eualus macilentus* and *E. stoneyi* were confined to Bathurst Inlet. Westward setting currents along the Beaufort Sea coast mediate against incursions by Pacific species but *Chionoecetes opilio, Hyas coarctatus alutaceus*, and a species of *Pagurus* seemed to have overcome this condition. The species in which the highest percentages of females presumably reproduced annually were *Spirontocaris phippsi, S. spinus*, and *S. septemcarinata.*

Received November 5, 1968

INTRODUCTION

COLLECTIONS BY THE CGS *Salvelinus* on the Canadian coast in southern Beaufort Sea and eastward to Wellington and Cambridge bays add to previously very scanty records of decapod crustaceans from this area.

The Canadian Arctic Expedition 1913–18 (Rathbun, 1919) obtained eight species of shrimps (Table 1) from dredge hauls and seals' stomachs at Bernard Harbour, Cockburn Point, and Stapylton Bay in Dolphin and Union Strait (Fig. 1 and 2). Anderson in 1910 obtained specimens of *Hyas coarctatus* at Langton Bay in Franklin Bay (Rathbun, 1919). Sivertsen (1932) estimated that 13 species of decapod crustaceans would occur in the Beaufort Sea. All his species except *Spirontocaris lilljeborgi* and *Eualus fabricii* were found in this collection but three additional species were obtained. Hart (1939) lists seven species of shrimps (Table 1) taken by the *St. Roch* in Dease Strait (Cape Alexander) and from Dolphin and Union Strait (capes Bexley and Krusenstern).

In a survey in 1962 by the Arctic Biological Station of the Fisheries Research Board of Canada (Squires, 1968) five decapod species were recorded from Wellington and Cambridge bays (Table 1).

The present paper records 15 species from coastal areas of the Canadian Arctic from Herschel Island to Wellington and Cambridge bays. Five of these species had not been reported from this area previously.

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TABLE 1. Decapod crustaceans reported in Canadian arctic coastal areas from Herschel Island to Wellington and Cambridge bays.

Species	Canadian Arctic expedition 1913–18	Anderson, 1910	*St. Roch* 1936–37	Fisheries Research Board, 1962	Present paper
Eualus fabricii	×	...	×	×	×
Eualus gaimardi	×	...	...	×	×
Lebbeus polaris	×	...	×	×	×
Lebbeus groenlandicus	×	...	×	...	×
Spirontocaris phippsi	×	...	×	...	×
Spirontocaris spinus	×	...	×	...	×
Sabinea septemcarinata	×	...	×	×	×
Sclerocrangon boreas	×	...	...	...	×
Hyas coarctatus	...	×	...	...	×
Argis dentata	...	...	×	×	×
Eualus macilentus	...	...	...	...	×
Eualus stoneyi	...	...	...	...	×
Sclerocrangon ferox	...	...	...	...	×
Pagurus sp.	...	...	...	...	×
Chionoecetes opilio	...	...	...	...	×

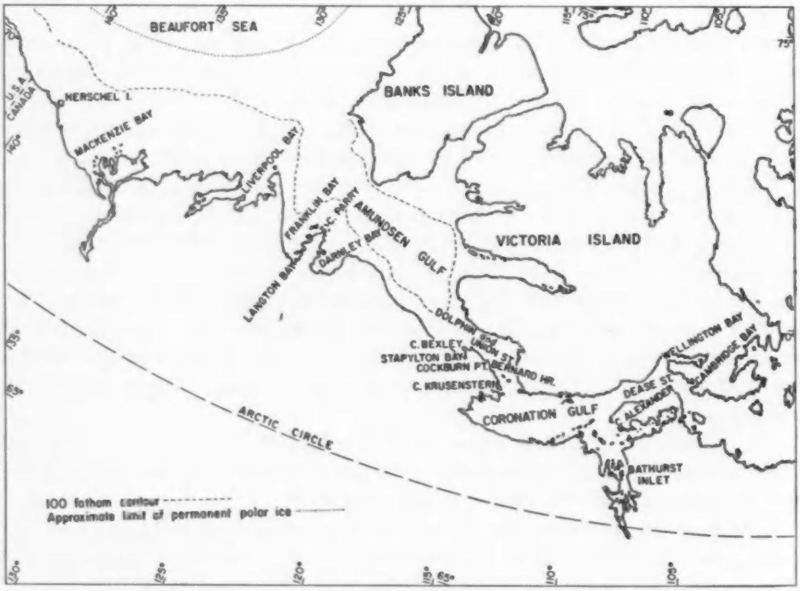
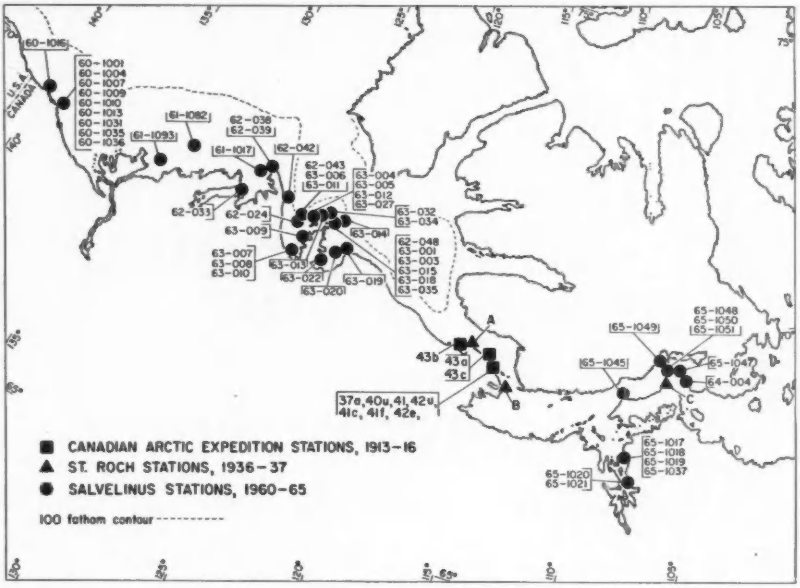

BEAUFORT SEA
BANKS ISLAND
VICTORIA ISLAND
U.S.A.
CANADA
HERSCHEL I.
MACKENZIE BAY
LIVERPOOL BAY
FRANKLIN BAY
C. PARRY
AMUNDSEN GULF
DARNLEY BAY
LANGTON BAY
DOLPHIN AND UNION ST.
C. BEXLEY
STAPYLTON BAY
COCKBURN PT.
BERNARD HR.
C. KRUSENSTERN
CORONATION GULF
DEASE ST.
C. ALEXANDER
WELLINGTON BAY
CAMBRIDGE BAY
BATHURST INLET
ARCTIC CIRCLE
100 fathom contour
Approximate limit of permanent polar ice
75°
70°
65°
140°
135°
130°
125°
120°
115°
110°
105°
100°

FIG. 1. Area of investigation and place names referred to from south Beaufort Sea to Cambridge Bay.


60-1016
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U.S.A.
CANADA
61-1082
61-1093
62-038
62-039
61-1017
62-042
62-043
63-006
63-011
63-004
63-005
63-012
63-027
62-033
62-024
63-009
63-032
63-034
63-014
62-048
63-001
63-003
63-015
63-018
63-035
63-007
63-008
63-010
63-013
63-022
63-020
63-019
A
43b
43a
43c
37a, 40u, 41, 42u, 41c, 41f, 42e,
B
65-1049
65-1048
65-1050
65-1051
65-1045
65-1047
64-004
C
65-1017
65-1018
65-1019
65-1037
65-1020
65-1021
■ CANADIAN ARCTIC EXPEDITION STATIONS, 1913-16
▲ ST. ROCH STATIONS, 1936-37
● SALVELINUS STATIONS, 1960-65
100 fathom contour ----------
140°
135°
130°
125°
120°
115°
110°
105°
75°
65°
69°

FIG. 2. Station numbers and locations of all captures of decapod crustaceans from south Beaufort Sea to Cambridge Bay. Canadian Arctic Expedition stations are numbered 37*a*–43*c* and *St. Roch* stations A–C.

METHODS AND HYDROGRAPHY RELATED TO THE COLLECTIONS

The CGS *Salvelinus* collected various invertebrates including decapod crustaceans in surveys for fish and benthos during the summers of 1960–65. Areas trawled extended from near the USA–Canada border to as far east as Cambridge Bay. Hauls were made near the coast in depths from about 10–60 m but some were from about 70 to 200 m deep. Near bottom temperatures were taken in 1963 and 1965 only. Generally depths greater than 30 m had temperatures of about –1.5 to –0.5 C but moderate temperatures of 2–6 C were found at shallower stations during July and August in Franklin and Darnley bays. Temperatures of about 2 C at 80 m, 4 C at 60 m, and 6 C at 30 m were found (Table 2).

Lengths of specimens referred to in this paper are carapace lengths only.

Family HIPPOLYTIDAE

Eualus fabricii (Kröyer)

Four specimens were taken: two ovigerous females 8 and 9 mm long from Cambridge Bay (Station 64-004; Fig. 3) and two adult males 6 mm long (with large vasa deferentia) from Bathurst Inlet (Station 65-1020). Depths recorded were 35 and 38 m and temperature –0.84 C.

Eualus gaimardi (H. Milne-Edwards)

With 582 specimens (144 ♂, 416 ♀, 22 not sexed) this was the second most abundant species in the collection. It appeared in catches throughout

TABLE 2. List of stations with numbers of specimens of decapod crustaceans collected from the Beaufort Sea and eastward to Wellington and Cambridge bays, 1960–65.

Station	Gear[a]	Species[b]								Depth (*m*)	Temp	Date	Remarks
		Eg	*Sph*	*Ssp*	*Lg*	*Lp*	*Sse*	*Ad*	*Hca*				
60–1001	O/T	9	...	...	1	...	6	...	...	...	...	July 26/60	
1004	Seine	3	...	...	...	...	...	...	...	...	...	Aug. 1/60	
1007	O/T	...	...	...	...	...	...	...	...	11	...	1/60	
1009	"	89	...	...	...	...	21	...	...	18–28	...	2/60	
1010	"	4	...	...	...	...	...	...	...	6–9	...	3/60	
1013	"	39	...	...	...	...	66	...	...	137	...	6/60	
1016	"	5	...	...	...	...	...	...	...	9–13	...	6/60	
1031	Pl. net	2	...	...	...	...	...	...	...	14	...	19/60	
1035	O/T	51	...	...	...	...	4	...	...	22–24	...	25/60	
1036	"	8	...	...	...	...	...	...	...	37–38	...	25/60	
61–1017	"	12	...	...	...	...	8	...	...	13	...	July 11/61	
1082	"	21	...	...	...	...	23	...	...	29–33	...	Aug. 19/61	
1093	"	1	...	...	...	...	1	...	...	14	...	Aug. 26/61	
62–033	"	10	...	...	...	...	...	1	...	18–27	...	Aug. 12/62	
038	Seine	1	...	...	...	...	...	...	...	0–2	...	17/62	
039	Seine	1	...	...	...	...	...	...	...	0–2	...	17/62	
042	O/T	1	...	1	...	...	12	...	...	68	...	21/62	
043	O/T	9	...	...	1	...	7	4	...	109	...	21/62	
048	Dredge	...	1	...	...	...	...	...	...	13–15	...	29/62	
63–001	Seine	...	1	...	...	...	...	...	...	1–9	...	July 19/63	
003	O/T	24	5	...	11	9	1	...	...	16	...	19/63	

63–004	"	19	...	1	...	...	16	1	...	30	−1.03		24/63	
005	"	16	...	...	...	...	5	1	...	95	−1.39		25/63	
006	"	24	...	...	...	...	...	...	...	178	−0.80		25/63	
007	"	33	30	27	35	1	1	22	198	18	4.98		31/63	+6 *S. boreas* +4 *Pagurus* sp.
008	"	3	...	...	...	...	1	1	1	20	5.05	Aug.	1/63	
009	"	1	...	...	1	...	1	...	...	14	4.90	"	"	
010	"	...	...	...	...	...	16	...	...	40–49	...	"	"	
011	"	96	...	...	...	...	3	1	...	180	−1.52		3/63	+6 *S. ferox*
012	"	...	...	...	...	...	...	...	...	80	−0.51		4/63	
013	"	2	...	...	1	...	...	...	...	19	...		4/63	
014	"	15	...	...	...	...	...	...	...	180	−1.27		3/63	
015	"	...	...	...	...	...	...	...	...	64	−0.52		8/63	
018	"	22	...	...	5	2	43	5	...	15	...		9/63	
019	"	...	...	...	...	...	1	3	...	80	2.19		12/63	
020	"	...	...	...	...	...	5	...	...	58	4.44		12/63	
022	"	14	...	...	...	...	32	...	...	30	5.83		13/63	
024	"	1	...	...	...	...	...	...	...	161	...		20/63	
027	"	20	...	...	...	...	105	3	1	33	5.66		20/63	
032	"	1	...	...	...	...	3	5	...	65	−0.94		28/63	+1 *C. opilio*

(*Continued*)

TABLE 2. List of stations with numbers of specimens of decapod crustaceans collected from the Beaufort Sea and eastward to Wellington and Cambridge bays, 1960–65. — (*Concluded*)

		Species[b]											
Station	Gear[a]	*Eg*	*Sph*	*Ssp*	*Lg*	*Lp*	*Sse*	*Ad*	*Hca*	Depth (*m*)	Temp	Date	Remarks
63–034	O/T	...	...	...	...	...	...	...	...	80	...	Sept. 5/63	+1 *C. opilio* (molted pereiopod shells only)
035	Longline	...	...	...	...	...	2	3	...	66	–0.94	5/63	
64–004	O/T	2	1	1	...	1	42	4	...	38	...	6/64	+2 *E. fabricii*
65–1017	"	...	...	...	...	...	5	...	...	10–35	–0.90	Aug. 11/65	
1018	"	...	...	...	...	...	44	6	...	10–40	–0.90	11/65	
1019	"	...	...	...	...	1	1	1	...	230	–1.18	11/65	
1020	"	...	...	...	...	...	17	1	...	35	–0.84	13/65	+2 *E. fabricii* +14 *E. macilentus* +1 *E. stoneyi*
1037	"	...	...	...	...	...	...	...	...	115	–1.04	18/65	+1 *E. macilentus*
1045	"	2	...	...	...	...	36	3	...	7	...	23/65	
1047	"	2	1	1	...	1	90	2	...	51	–1.27	27/65	
1048	"	5	...	6	...	...	75	5	...	60	–1.24	30/65	
1049	"	4	...	6	...	8	29	...	...	47	–1.05	30/65	
1050	"	10	...	...	...	...	47	...	...	51	–1.27	Sept. 1/65	
1051	"	...	...	...	...	...	47	...	...	40	–0.60	1/65	
Total		582	39	43	55	23	816	72	200				

[a] O/T = otter trawl; Pl. net = plankton net.

[b] *Eg* = *Eualus gaimardi*; *Ssp* = *Spirontocaris spinus*; *Sph* = *S. phippsi*; *Lg* = *Lebbeus groenlandicus*; *Lp* = *L. polaris*; *Sse* = *Sabinea septemcarinata*; *Ad* = *Argis dentata*; *Hca* = *Hyas coarctatus alutaceus*.

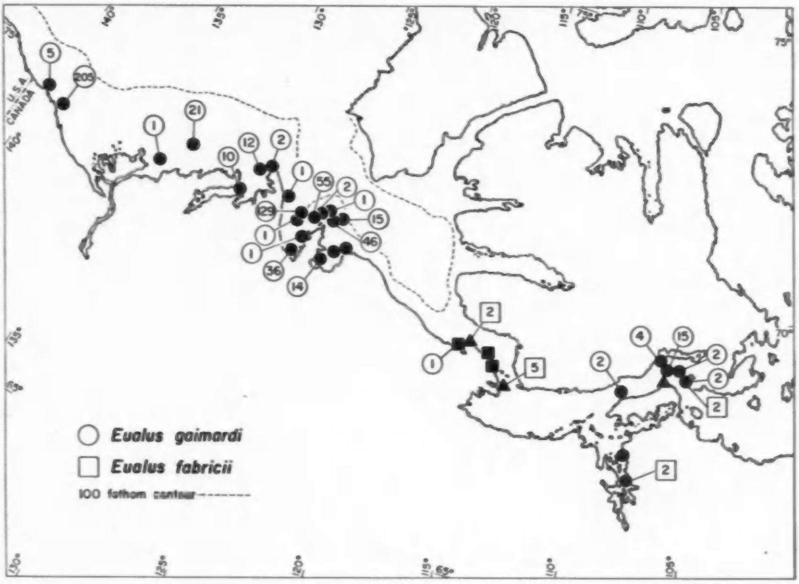


FIG. 3. Recorded distributions of *Eualus fabricii* and *E. gaimardi* from south Beaufort Sea to Cambridge Bay. Numbers of specimens taken at each of the stations (Fig. 2) are shown.

the area but in greatest numbers near Herschel Island and in Franklin Bay (Fig. 3). Depths recorded were 0–180 m and temperatures −1.52 to 5.83 C (Tables 2 and 4).

The percentage (35%) potentially ovigerous (some ovigerous and some with eggs ready to be laid) was low (Table 3) indicating poor adaptation and probably biennial spawning under arctic conditions (Squires, MS, 1965, 1967b). However, specimens were larger, generally, than those taken in other areas in the arctic except those from Hudson Bay, where they were about the same size (Squires, 1957, MS, 1965, 1967a, and 1968). First maturity in males and females (smallest with large vasa deferentia or carrying eggs) was at 7 and 8 mm (Table 3), appreciably smaller than in other arctic areas and 1 mm less than in Hudson Bay. No eye pigment was present in eggs examined from specimens obtained from July 26 to September 1.

Average lengths of males and females were 10 mm (5–15 mm) and 12 mm (5–22 mm) (Fig. 9; Table 4).

Eualus macilentus (Kröyer)

Fifteen specimens (7 ♂, 8 ♀) were taken from Stations 65-1020 and 65-1037 in Bathurst Inlet (Fig. 4). Depths were 35 and 115 m (14 specimens were from 35 m), and temperatures −0.84 and −1.04 C.

TABLE 3. Maturity of male and female decapod crustaceans collected from the Beaufort Sea and eastward to Wellington and Cambridge bays, 1960–65. CL = carapace length; Vd = vas deferens.

		Males			Females				
					Nonovigerous				
Species	Juveniles	Vd small	Vd large	CL at 1st maturity (*mm*)	Small ova	Large ova	Ovigerous	Smallest size (CL) taken with large Vd or ova (*mm*)	Potentially ovigerous annually (%)
Eualus gaimardi	8	73	71	7	270	2	144	8	35
Spirontocaris phippsi	...	...	2	5	8	7	22	6	78
S. spinus	...	5	9	6	9	10	10	8	69
Lebbeus groenlandicus	...	3	17	6	18	4	3	12	28
L. polaris	...	1	7	8	8	4	1	10	38
Sabinea septemcarinata	2	65	97	7	307	90	248	13	53
Argis dentata	2	16	6	8	34	6	7	21	30
Hyas coarctatus alutaceus	...	13	2	22	15	...	1	51	...

TABLE 4. Summary of depths, temperatures, and sizes of male and female decapod crustaceans collected from the Beaufort Sea and eastward to Wellington and Cambridge bays, 1960–65. CL = carapace length.

Species	Males			Females			Depth range (*m*)	Temp range (*C*)
	No. examined	CL (*mm*)		No. examined	CL (*mm*)			
		Range	Avg		Range	Avg		
Eualus gaimardi	144	5–15	10	416	5–22	12	0–180	-1.52–5.83
Spirontocaris phippsi	2	5	5	37	5–10	7	1–51	-1.27–4.98
S. spinus	14	5–15	9	29	5–16	11	18–68	-1.27–4.98
Lebbeus groenlandicus	23	6–13	10	32	5–26	11	14–109	4.90–4.98[a]
L. polaris	9	7–10	9	13	5–12	10	15–230	-1.27–4.98
Sabinea septemcarinata	164	6–18	10	644	6–20	14	7–180	-1.40–5.83
Argis dentata	22	6–23	11	47	9–28	17	7–230	-1.40–5.66
Hyas coarctatus alutaceus	89	6–61	12	117	6–51	12	18–33	4.98–5.66

[a] From 14 and 18 m only.

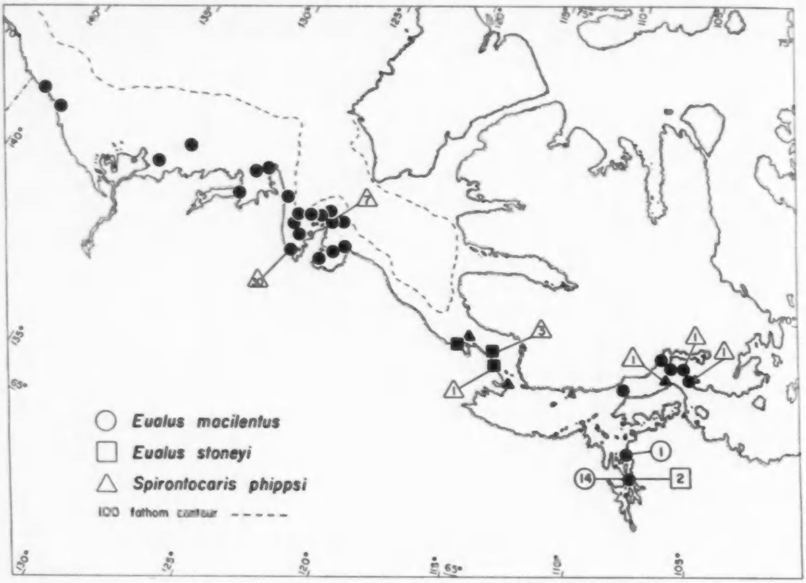


FIG. 4. Recorded distributions of *Eualus macilentus*, *E. stoneyi*, and *Spirontocaris phippsi* from south Beaufort Sea to Cambridge Bay. Numbers of specimens taken at each of the stations (Fig. 2) are shown.

The rostrums of some of these specimens were somewhat more attenuate than usual for the species with a relatively low keel in 5 out of 11 specimens. However, rostral formulae, $\frac{13\text{–}16}{2\text{–}3}$, were typical of the species. Four females, 6–8 mm long, and 6 males, 6–9 mm long, were mature.

The occurrence of *E. macilentus* many hundreds of miles from other recorded locations for this species suggests that it may form an isolated population here. The nearest records were obtained in Hudson Bay, Ungava Bay, and Greenland to the east (Squires, 1966) and Point Barrow, Alaska (one questioned specimen) to the west (MacGinitie, 1955). The depth of 35 m is well within the range of many intervening stations to the eastward where collections of decapods were made in recent years (Squires, MS, 1965, 1967a, 1968).

Eualus stoneyi Rathbun

Two specimens (2 ♀) were taken from stations 65-1020 and 65-1021 in Bathurst Inlet (Fig. 4). The depth recorded was 35 m and temperature −0.84 C.

The rostral formula, $\frac{7\text{–}8}{0\text{–}1}$, was typical for the species. Both females, 3 and 4 mm long, had immature gonads.

As in *E. macilentus* above, this species probably forms an isolated population in this area. The nearest recorded locations are at Nutak on the northern Labrador coast (Squires, MS, 1965) to the east and in the Bering Sea to the west (Rathbun, 1904).

Spirontocaris phippsi Kröyer

Thirty-nine specimens (2 ♂, 37 ♀) were taken in Franklin, Darnley, and Cambridge bays (Fig. 4). Depths were 1–51 m and temperatures −1.27–4.98 C at times of capture (Tables 2 and 4).

Lengths in males and females at first maturity were 5 and 6 mm (Table 3), the same as from other areas in the Arctic. Apparently 78% of the adult females would spawn annually (Table 3). Average length in females was 7 mm (5–10 mm) (Fig. 9; Table 4).

Spirontocaris spinus (Sowerby)

Forty-three specimens (14 ♂, 29 ♀) were taken from Franklin, Darnley, Wellington, and Cambridge bays (Fig. 5). Depths were 18–68 m and temperatures −1.27–4.98 C (Tables 2 and 4).

All the females were similar to Rathbun's *S. arcuata* but the males were like *S. spinus*. The accessory spinules of the rostrum were more numerous than usually found in *S. spinus* in most specimens but this is an expected variation such as occurs in many species of decapod crustaceans in arctic waters. (Squires (1957) gives several examples of numbers in rostral spine formulae greater than quoted by Rathbun (1929).) I am therefore assigning these specimens to *S. spinus* rather than *S. arcuata*. Rathbun (1919) reported one specimen of *S. arcuata* from Cockburn Point. MacGinitie (1955) reported eight specimens of *S. arcuata* from Point Barrow, Alaska. The sex of these specimens was not indicated.

Length at first maturity was 6 mm in males and 8 mm in females (Table 3), the same as in Foxe Basin but larger than in Hudson or Ungava bay (Squires, 1967a). The percentage (69%) ovigerous annually was relatively high for this species, perhaps indicating annual spawning of most adults (Table 3). Average lengths in males and females were 9 mm (5–15 mm) and 11 mm (5–16 mm) (Fig. 9; Table 4).

Lebbeus groenlandicus (Fabricius)

Fifty-four (23 ♂, 31 ♀) specimens were taken in Franklin and Darnley bays and near Herschel Island (Fig. 5). Depths recorded were 14–109 m and temperatures 4.90–4.98 C (at depths of 14 and 18 m only) (Tables 2 and 4).

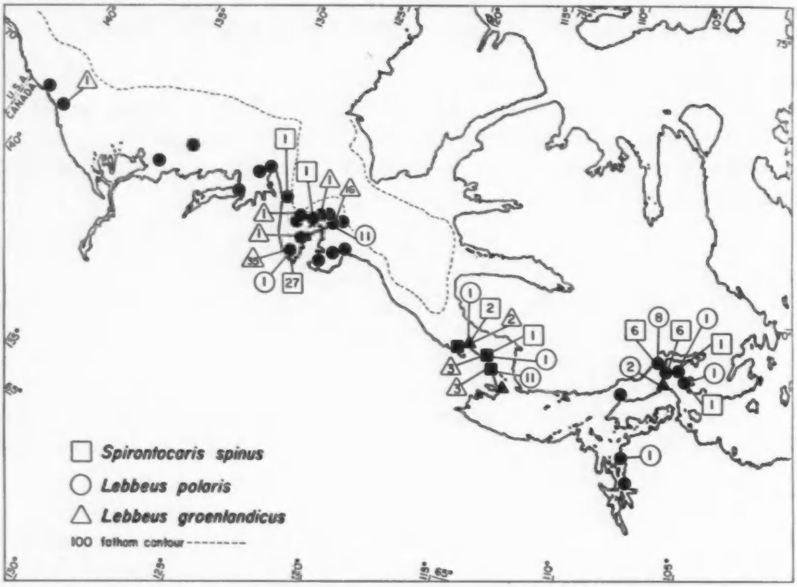


FIG. 5. Recorded distributions of *Spirontocaris spinus*, *Lebbeus polaris*, and *L. groenlandicus* from south Beaufort Sea to Cambridge Bay. Numbers of specimens taken at each of the stations (Fig. 2) are shown.

Males and females when first mature were 6 and 12 mm long. They were smaller than in the Queen Elizabeth Islands (Squires, 1968) and in Hudson and Ungava bays (Squires, 1967a). The low percentage of specimens (28%) (Table 3) that would be ovigerous indicated poor adaptation in the area, or may be misleading because of the small number of specimens. Average lengths in males and females were 10 mm (6–13 mm) and 11 mm (5–26 mm) (Fig. 9; Table 4).

Lebbeus polaris (Sabine)

Twenty-three specimens (9 ♂, 13 ♀, 1 not sexed) were taken in Franklin, Darnley, Cambridge, and Wellington bays and in Bathurst Inlet (Fig. 5). Depths were 15–230 m and temperatures −1.27–4.98 C (Tables 2 and 4).

Length at first maturity was 8 mm in males and 10 mm in females (Table 3) and appeared to be similar to those from other arctic areas (Squires, 1967a). About 38% would be ovigerous annually (Table 3). Average lengths in males and females were 9 mm (7–10 mm) and 10 mm (5–12 mm) (Fig. 9; Table 4).

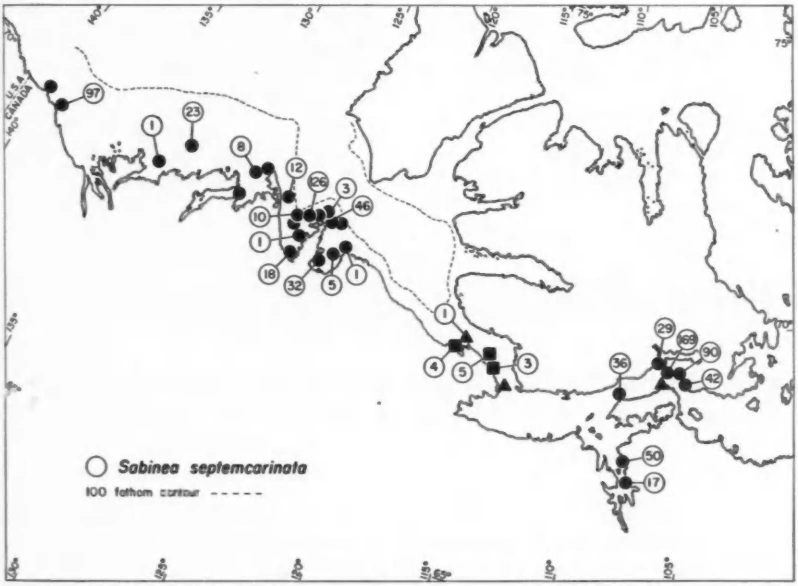
Family CRANGONIDAE

Sabinea septemcarinata (Sabine)

With 816 specimens (164 ♂, 644 ♀, 8 not sexed) this was the most abundant species in the collection. It was taken at almost all stations from Herschel

Island to Cambridge Bay (Fig. 6). Depths were 7–230 m and temperatures -1.52–5.83 C (Tables 2 and 4).

Length at first maturity was 7 mm in males and 13 mm in females (Table 3). These sizes were smaller than those of Queen Elizabeth Islands and Hudson Bay specimens (Squires, 1967a, 1968) but rather similar to those from Foxe Basin (Squires, MS, 1965). Of the adult females, 53% would be ovigerous annually (Table 3). About 20% of the ovigerous females had eggs with early eyed embryos when taken during August 1 to September 6. Some (five) had recently hatched eggs as late as August 30. Average lengths of males and females were 10 mm (6–18 mm) and 14 mm (6–20) (Fig. 9; Table 4).


140°
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10
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18
32
5
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36
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169
90
42
50
17
135°
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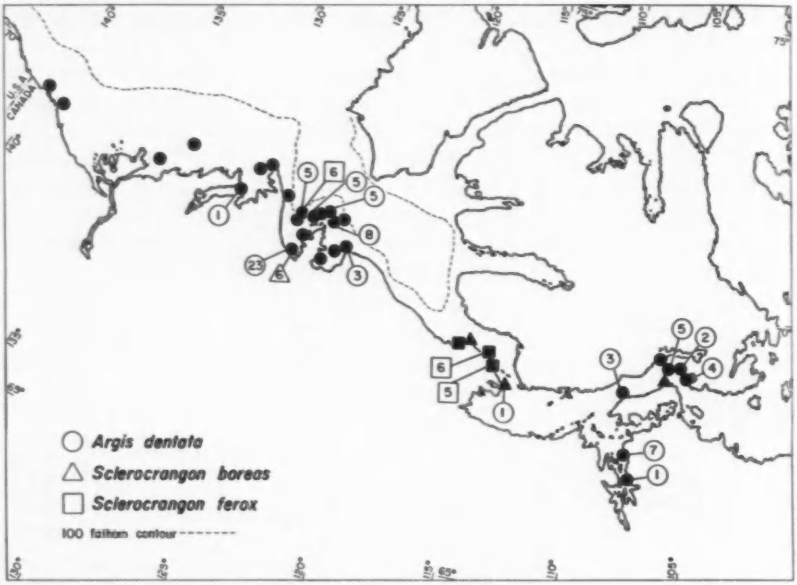
○ Sabinea septemcarinata
100 fathom contour -----
140°
125°
120°
115° 65°
110°
105°

FIG. 6. Recorded distributions of *Sabinea septemcarinata* from south Beaufort Sea to Cambridge Bay. Numbers of specimens taken at each of the stations are shown (Fig. 2).

Argis dentata Rathbun

Seventy-two specimens (22 ♂, 47 ♂, 3 not sexed) were taken in Liverpool, Franklin, Darnley, Wellington, and Cambridge bays, Dease Strait, and Bathurst Inlet (Fig. 7; Table 2). Depths were 7–230 m and temperatures -1.27–5.66 C (Table 4).

Length at first maturity was 8 mm in males and 21 mm in females (Table 3). These figures compare with 15 and 20 mm for Frobisher Bay specimens and 9 and 14 mm for Hudson Bay specimens (Squires, 1962, and 1967a). The low percentage (30%) ovigerous (Table 3) annually suggests biennial


U.S.A.
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○ Argis dentata
△ Sclerocrangon boreas
☐ Sclerocrangon ferox
100 fathom contour -------

FIG. 7. Recorded distributions of *Argis dentata*, *Sclerocrangon boreas*, and *S. ferox* from south Beaufort Sea to Cambridge Bay. Numbers of specimens taken at each of the stations (Fig. 2) are shown.

spawning (Squires, 1967b). Average lengths of males and females were 11 (6–23 mm) and 17 mm (9–28 mm) (Fig. 9; Table 4).

Sclerocrangon boreas Phipps

Six specimens (2 ♂, 4 ♀,) were taken at Station 63-007 in Franklin Bay (Fig. 7). The depth was 18 m and the temperature 4.92 C (Table 2).

Both males were adult at 15 mm long and the females were all immature at 8–12 mm long.

Sclerocrangon ferox G. O. Sars

Only six specimens (1 ♂, 5 ♀) were taken at Station 63–011 in Franklin Bay (Fig. 7). The depth was 180 m and the temperature –1.52 C (Table 2).

The male was adult at 20 mm, three females immature at 20 and 21 mm, one female with large ova at 31 mm, and one female ovigerous at 30 mm.

Family PAGURIDAE

Pagurus sp.

Only four specimens were taken at Station 63-007 in Franklin Bay (Fig. 8). The depth was 18 m and the temperature 4.98 C (Table 2). The specimens

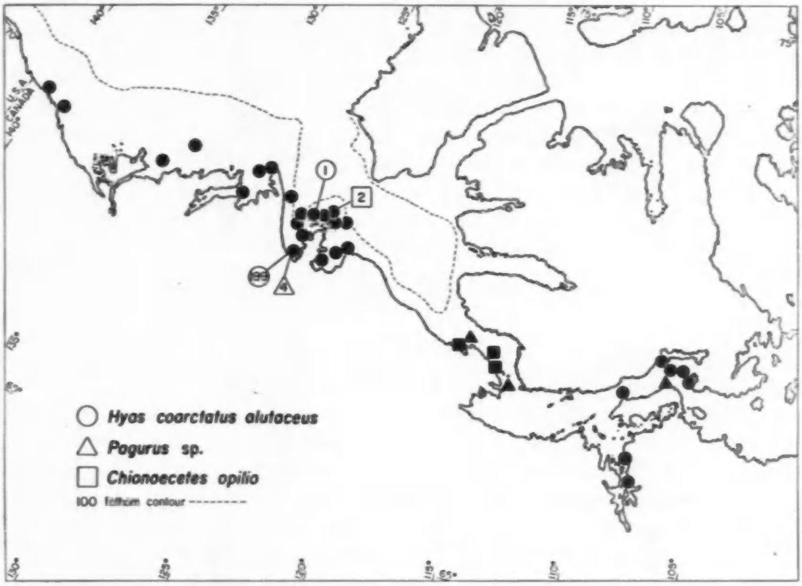

FIG. 8. Recorded distributions of *Hyas coarctatus alutaceus*, *Pagurus* sp., and *Chionoecetes opilio* from Beaufort Sea to Cambridge Bay. Numbers of specimens taken at each of the stations (Fig. 2) are shown.

were all juvenile at 3 mm and difficult to identify. The anterior portion of the third sternite, however, was too high for *P. pubescens* (Squires, 1964a) and could belong to *P. trigonocheirus*.

Family MAJIDAE

Hyas coarctatus alutaceus Brandt

There were 199 specimens (89 ♂, 110 ♀) taken at stations 63-007, 63-008, and 63-027 in Franklin Bay (Fig. 8). Depths were 18, 20, and 33 m, and temperatures 4.98, 5.05, and 5.66 C (Table 2). At Station 63-007 the 197 specimens taken were juvenile males and females from 6 to 20 mm and 6 to 19 mm long, respectively. In addition, one adult male of 61 mm was taken at Station 63-008 and one ovigerous female of 51 mm at Station 63-027.

Chionoecetes opilio (O. Fabricius)

Only two specimens (1 ♂, 1 not sexed) were collected off Cape Parry at 65 and 80 m (Fig. 8) and at a temperature of −0.94 C.

One comprised the molted shell of the legs only (merus of second pereiopod = 10.5 mm long) and the other an intact adult male 71 mm long (merus of second pereiopod = 9.2 mm long).

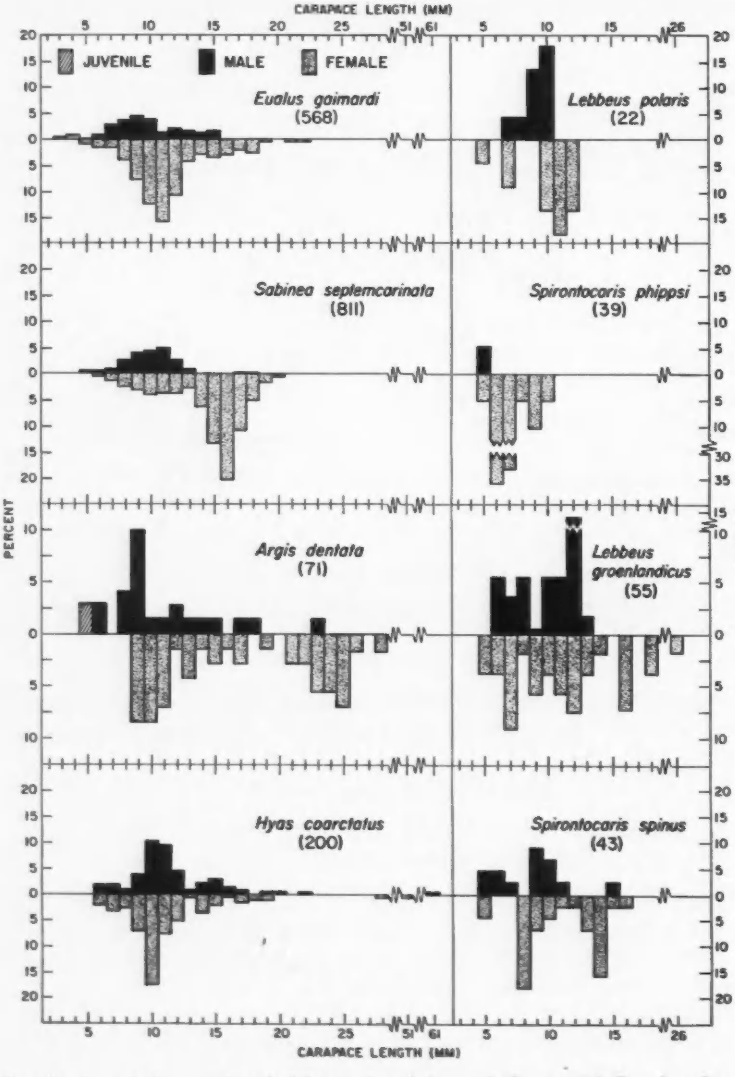

FIG. 9. Length frequencies of eight species of decapods from south Beaufort Sea to Cambridge Bay. The numbers of specimens are in parentheses.

Rathbun (1919) refers to correspondence stating that R. M. Anderson had collected this species at Herschel Island. The present record extends its range eastward to Cape Parry.

STOMACH CONTENTS

Eualus macilentus and *E. gaimardi* appeared to feed on phytobenthos and detritus more than other species (Table 5) but, generally, stomach contents of species from this area add little new information on feeding in the species collected. *Spirontocaris phippsi* had a higher than usual incidence of feeding on crustaceans (mostly ostracods) and a usual preference for foraminiferans as shown in previous records of stomach contents (Allen, 1962; Squires, MS, 1965). *Spirontocaris spinus* fed on small clams and small crustaceans more than usual. Primarily preying on small crustaceans were the large species *Sclerocrangon*, *Argis*, *Sabinea*, and *Lebbeus*.

DISCUSSION

This paper is one of seven on decapod Crustacea of Canadian Arctic waters including Ungava Bay (Squires, 1957, 1965), Frobisher Bay (Squires, 1962), Fox Basin, etc. (Squires, MS, 1965), Hudson Bay (Squires, 1967a), and Queen Elizabeth Islands (Squires, 1968). Records of numbers of specimens at the depths sampled indicate discontinuities in distribution of species such as *Eualus macilentus* and *E. stoneyi*, and the widespread occurrence in the Arctic of species such as *E. gaimardi* and *Sabinea septemcarinata*.

Zoogeographical considerations — von Hofsten (1916) and Stephensen (1935) classified the species of decapods according to their distribution and occurrence at low temperatures as high-arctic, panarctic, arctic-boreal, etc. Of their three high-arctic species, *Bythocaris payeri* occurs as far south as the Grand Bank (about 45°N) and *Sclerocrangon ferox* at about 57°N off Labrador, both from temperatures above 0 C on the deep continental slope (Squires, 1966). *Sclerocrangon ferox*, however, does occur in shallow water in the Queen Elizabeth Islands (Squires, 1968) and in the Beaufort Sea, so that it is essentially more arctic than the other two. Also, among their boreal species, Hofsten and Stephensen list *Hyas coarctatus*. But this species takes the place of *Hyas araneus* in arctic areas north and west of Cape Chidley (Squires, 1965), and its subspecies, *alutaceus*, as far west as the Beaufort Sea in the present collection. It does not seem reasonable to say that such species are accidentals in arctic waters when they are apparently self-propagating there. Also, all species examined in the present collection are found in arctic and subarctic as well as boreal regions as defined by Dunbar (1951). To classify a species as arctic, therefore, would depend on whether it were found only where predominantly arctic waters (with temperatures close to 0 C most of the year) were present. Boreal species would be in predominantly Atlantic waters with temperatures of about 5 C (±2 C) most of the year. Species such as *Eualus macilentus*, *E. gaimardi*, and *Sabinea septemcarinata* I would therefore classify as arctic whereas *Pandalus borealis*, *Pagurus arcuatus*, and *Homarus americanus* would be boreal. Species such as *Lebbeus polaris* would be difficult to classify since they have been taken in a comparatively wide range of temperatures as well as in boreal and arctic regions.

TABLE 5. Stomach contents of decapod crustacean species (per cents of the stomachs examined that contained the various items) from Beaufort Sea and eastward to Cambridge Bay, 1960–65.

Species	E[a]	Ph	C	Ost	P	F	Ss	Ga	Pe	Am	Total examined
Eualus fabricii	100	...	...	...	...	...	...	...	...	...	2
E. gaimardi	56	26	21	1	1	1	1	...	...	...	477
E. macilentus	86	14	...	...	...	14	...	...	...	...	7
Spirontocaris phippsi	13	16	63	50	...	37	...	...	2	...	38
S. spinus	35	3	41	...	...	19	...	...	43	...	37
Lebbeus groenlandicus	62	8	22	...	...	6	...	...	2	...	50
L. polaris	80	13	17	...	...	...	...	...	...	...	15
Sabinea septemcarinata	87	4	7	...	2	...	1	...	...	...	678
Argis dentata	78	2	22	...	...	...	...	2	6	2	54
Sclerocrangon boreas	50	...	50	...	...	...	...	...	...	...	6
S. ferox	100	...	...	...	...	...	...	...	...	...	4
Pagurus pubescens	100	...	...	...	...	...	...	...	...	...	4
Hyas coarctatus	27	8	50	19	8	8	...	...	...	...	26
Chionoecetes opilio	...	...	100	...	...	...	...	...	...	...	1

[a]E = empty; Ph = phytobenthos; C = crustacean; Ost = ostrocod; P = polychaete; F = foraminiferan; Ss = sponge spicules; Ga = gastropod; Pe = pelecypod; Am = amphipod.

Influence of currents in the Beaufort Sea — Currents move westward along the Canadian and Alaskan shores in the Beaufort Sea until they reach Point Barrow, Alaska, where they turn northward. West of Point Barrow the currents flow eastward but join the northward-flowing current off Point Barrow (Grosvenor and Darley, 1963). As a transitional area between the Chukchi and Beaufort seas it is not surprising, therefore, that the Point Barrow area has some decapod species from the Pacific such as *Pandalus borealis*, *Eualus suckleyi*, *Heptacarpus flexus*, *Crangon communis*, *Argis lar* (Squires, 1964b), *Pagurus splendescens*, and *Paralithodes camtschatica* (MacGinitie, 1965) although these species are apparently not present in the Beaufort Sea. However, *Chionoecetes opilio*, *Pagurus trigonocheirus*, and *Hyas coarctatus alutaceus* may have spread into this area within comparatively recent time. Their larvae presumably spend a longer time in the plankton than caridean larvae and they could have been carried (albeit under ice) in the eastward currents forming the large outer part of the gyre in the Beaufort Sea. The current system would tend to set up counter gyres in Franklin and Darnley bays, where these species would be encouraged to settle and where they were taken by the present collections. The large numbers of juvenile *H. coarctatus alutaceus* taken indicate a nursery area for this species in Franklin Bay.

Bioeconomics of decapod crustaceans — Decapod species occupy an important place in the bioeconomics of the Arctic, where they form part of the food of seals, beluga whales, murres, fishes, etc. These decapods feeding on phytobenthos, detritus, small crustaceans, etc., are close to the primary part of the food chain in the sea (Table 5). Even in the high Arctic they apparently reproduce successfully and some species may produce a moderately high yield as shown by their abundance in hauls. However, none appears sufficiently abundant for even a short summer fishery but if small traps could be used effectively for their capture, they would have value as a subsistence item for human beings in the Arctic.

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NOTES

Isopropanol–Water Mixtures for the Production of Fish Protein Concentrate From Atlantic Herring (*Clupea harengus*)

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The efficiency with which isopropanol extracts fat and other materials from minced round herring depends upon the water to solvent ratio in the liquid phase. Optimum dilutions for maximum extractive efficiency are determined. There is a considerable difference in the efficiency with which isopropanol–water mixtures extract fat from herring and cod.

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Because of the potential value of herring as a raw material for fish protein concentrate (FPC) there has been a growth of interest in the use of herring for this purpose. The Halifax process for the production of FPC as described by Power (1962) and Idler (1967) was essentially designed for the use of lean fish as raw material. The process was empirically adapted to fatty fish material (herring particularly) by Power (1964). However, since there is a considerable difference in the chemical composition of the fat of gadoid and clupeid fishes (Lovern, 1961; Linko, 1967), it was felt that a systematic exploration of the efficiency of various isopropanol (IPA)–water mixtures for the production of FPC would be of value for the further development of the Halifax process.

Materials and methods — The herring used for this study was caught in August off Halifax harbour. The lot consisted of specimens uniform in size and fully developed sexually at the prespawning stage. The fish were placed into polyethylene bags and immediately frozen. The bags were kept at −40 C for a month before used, then the frozen herring was ground twice in an industrial meat grinder and after mixing the still frozen and granular material was weighed in 100-g portions into glass-stoppered flasks. Calculated volumes of reagent grade IPA were added to each flask so that the alcohol would form, with the natural water of the sample, mixtures containing in the liquid phase 10, 20, 30, 40, 50, 60, 70, 80, 90% water (v/v). The total volume in each flask, except for the 10% dilution, was adjusted to 300 ml by adding corresponding IPA–water mixtures. Each flask was boiled with reflux for 15 min. The contents, still hot, were filtered through a sintered glass plate, rinsed twice with 25 ml of the same hot solvent as used in the extraction. The filter cake, usually containing 50–60% of liquid was returned into the flask for second extraction with 150 ml of the same IPA–water mixture. In the third and last extraction

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TABLE 1. Gross chemical composition of Atlantic herring in a pre-spawning stage (average sample of 24 whole fish), in per cent of sample.

	%
Moisture	68.2
Total fat	12.3
Phospholipids	2.1
Total protein	17.0
Extractable protein	5.6
Nonprotein nitrogenous compounds	2.5
Minerals (ash)	2.3
Bones, scales	4.2

anhydrous IPA was used. The second and third extractions were performed in the same manner as the first extraction except for the solvent–water ratio used.

The filtrates of the three extractions were analyzed separately. First the solvent and the major part of water were eliminated in a rotary vacuum evaporator, then the lipids were eluted with ethyl ether. The aqueous residue containing the nonlipid extractives or so-called water-solubles was evaporated and dried to constant weight. The protein content in the water-solubles was determined by the biuret method. The ethyl ether–lipid solution was also evaporated and dried to constant weight. The phospholipid fraction in the total fat was determined either by chromatography on 200-mesh activated silica or by precipitation with dry acetone. The filter cakes were dried in vacuum at 50–60 C to constant weight. With some samples it was necessary to grind the sample before the constant weight could be reached. The dried material, besides the protein, contains bones and scales. The total fat of the raw material was determined by exhaustive extractions alternatively using IPA containing 30% water and dry solvent as described before. The ashing was made in an electric furnace at 550–600 C. The moisture of the raw material was determined by two methods — azeotropic distillation and direct drying under vacuum at 50–60 C. The results were identical, 68.2%.

Results and discussion — It was demonstrated previously (Dambergs, 1959, 1963) that cod fillets could be completely defatted by three consecutive extractions with aqueous IPA at room temperature. This method applied to a whole herring did not produce the same results. Emulsions hampering filtrations were formed and when the aqueous IPA ceased to extract fat, considerable amounts of additional fat were extracted with less aqueous or anhydrous IPA. Complete extraction of fat could be achieved by the alternate use of aqueous and anhydrous IPA. When boiling solvents were used, the yields of fat increased and the filtrations became more efficient.

The results of fat extractions with various IPA–water mixtures are shown in Fig. 1. It can be seen that the first extraction with aqueous IPA produces continuously decreasing amounts of fat as the solvent–water ratio is decreased. IPA containing 10% water extracts 90% of the fat present in the sample, whereas IPA containing 50% water extracts only half of the fat and the yields decreased with further dilution.

The second extraction with aqueous IPA in the case of herring is inefficient. This has been already demonstrated by Power (1964).

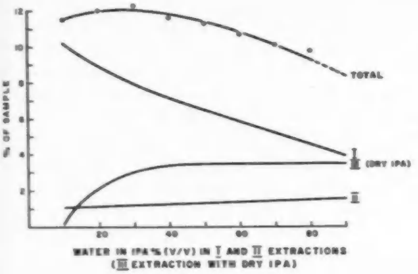

FIG. 1. Lipids extracted from whole herring with various IPA–water mixtures. ○ — ○ = sum of lipids extracted in I + II + III extractions; I = lipids extracted in the first extraction; II = lipids extracted in the second extraction; III = lipids extracted in the third extraction.

The third extraction, in which dry IPA is used, is more efficient and this indicates that it is advantageous to use less aqueous or anhydrous IPA in both the second and the final extraction.

Very little is known about the phospholipid content in whole herring. The published results usually refer to oils obtained from the skeletal muscle only. Lovern (1961) gives as a range for the phospholipid concentration in Atlantic herring muscle 0.7–0.8% and Linko (1967) for fillets of Baltic herring gives the range 0.6–1.5%. However, it is known that the viscera, sexual products, and dark meat have higher concentrations of phospholipids than the fillet. The oils extracted in experiments in which nearly perfect extraction was achieved were analysed for their content of phospholipids. Two methods — the chromatography on activated silica and the precipitation with dry acetone — were used. The average phospholipid content for this batch of sexually ripe Atlantic herring was 2.1% of the body weight. Since no particular protective measures against oxidation or hydrolysis of the phospholipids were taken, this value can be considered as a minimum value.

The phospholipids were again determined in an identical manner 3 months later. The herring had accomplished the spawning and were feeding intensively. The phospholipid content was 2.9% of the body weight. It is noteworthy, that in the process of fat extraction with ethyl ether from the crude extract obtained from herring with aqueous solvents stable emulsions are always formed. They separate as an intermediate layer between the ethyl ether–lipid solution and the lower aqueous layer of the water-solubles. Some of the emulsions were isolated for analysis. It was impossible to extract all the lipids from these aqueous emulsions with ethyl ether alone. However, upon addition of anhydrous solvent (IPA, acetone, or ethanol), the solids of the emulsion settled out and could be completely defatted. After drying the solids were in the form of a cream-colored powder which gave a strong biuret reaction when dissolved in alkali. The fat extracted from the emulsion gave a positive reaction for phosphorus. This indicates that some of the phospholipids of herring are extracted by aqueous IPA in the form of stable lipoproteins which seem to be dissociated only by anhydrous solvents.

It is seen from Fig. 2 that the amount of the nonlipid materials extracted increases sharply with the dilution of the IPA. A maximum is reached in the

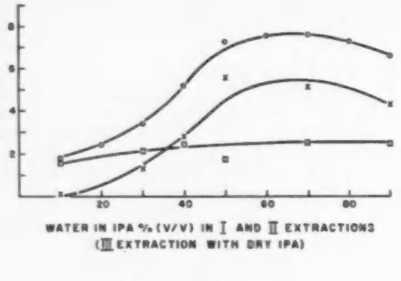

FIG. 2. Water-soluble extractives and the soluble proteins extracted from whole herring with various IPA–water mixtures. ○ — ○ = total water-soluble extractives; × — × = extractable proteins in the total extract; □ — □ = nonprotein introgenous extractives.

region of 50–70% water in IPA when 7.6% of the weight of the sample or 24% of the dry weight of the sample is extracted besides the lipids.

The protein content in the nonlipid fraction of the extract is negligible when the IPA contains 10% water, but increases sharply with dilution reaching a maximum when alcohol containing 50–70% is used. The difference between the total nonlipid extractives and the proteinaceous extractives are the true water-solubles. They are low molecular weight compounds with high potential to affect adversely the quality of the FPC. To improve the storage qualities of the FPC it is important to extract the major part of these compounds. At the same time the extraction of the soluble proteins must be kept to the minimum. A compromise is achieved when IPA containing about 30% water is used in the first extraction. At this concentration about 70% of the fat, 84% of the true water-solubles, and only 25% of the total soluble proteins are extracted. The second and eventually third extraction with less aqueous or anhydrous IPA will take out the remaining fat and moisture and only negligible amounts of nonfat materials.

The yield of FPC decreases rapidly with a decrease in the alcohol–water ratio as shown in Fig. 3. As the amount of material extracted, especially protein, increases the yield is reduced. The maximum extractive efficiency and the minimum yield of FPC is obtained with a solvent containing 50–60% water.

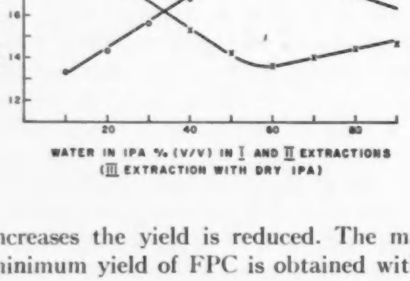

FIG. 3. Total extractives (lipids + water-solubles) and the proteinaceous residue obtained in extractions of whole herring with various IPA–water mixtures. ○ — ○ = total extractives; × — × = proteinaceous residue (FPC).

Conclusions — It is desirable to extract the maximum amount of lipid and water-solubles and the minimum amount of soluble protein in processing FPC. As the maximum amount of lipid from herring is extracted by dry IPA, the maximum amount of water-solubles is extracted by IPA containing 65% water, and the minimum amount of protein is extracted by dry IPA, the selection of a IPA–water mixture for extracting herring must be a compromise. Initially herring contains approximately 65% water. This must be utilized as part of the solvent for the first extraction. The addition of sufficient IPA to result in a 70:30 IPA:water mixture gives a slurry of viscosity low enough that it can be stirred and pumped and it is reasonably effective in extracting lipid. The amount of protein extracted is not great and although the extractive efficiency of this mixture is not optimum for water-solubles it is a reasonable compromise. The second and third extractions should employ anhydrous IPA. These factors indicate that the industrial method to produce FPC from whole herring with IPA as a solvent preferably should be a continuous process.

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Isopropanol–Water Azeotrope as Solvent in the Production of Fish Protein Concentrate from Herring (*Clupea harengus*)

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In the process of the production of fish protein concentrate (FPC) the isopropanol is recovered, most economically, in the form of an aqueous azeotrope. It has been demonstrated that the azeotrope can be used effectively for processing FPC avoiding the necessity of dehydration.

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ISOPROPANOL, or isopropyl alcohol (IPA) as a fat extractant was first used in the cotton industry (Harris et al., 1947). As a defatting agent of fisheries raw material it was first used by Guttman and Vandenheuvel (1957) in Halifax

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Laboratory of the Fisheries Research Board of Canada. The first fish protein concentrate (FPC) obtained was bland and practically colorless. However, the product lacked storage stability and processing difficulties were encountered. Further studies revealed that the main factor governing the efficiency of the extraction of both the fat and the water solubles is the ratio of water to the IPA in the liquid phase during the operation (Dambergs, 1959). Samples of cod FPC of various degrees of purity, obtained in these and later experiments, were kept in screw-capped jars at room temperature and periodically inspected for quality. The samples which contained more than 0.05% residual fat deteriorated in a few weeks. The next ones to deteriorate were samples that contained less than 0.05% fat but had more than 4% residual water-soluble material. This amount approximately corresponds to 25% of the initial amount of the water-solubles. Samples containing less than 0.05% fat and less than 4% water-solubles remained stable for 10 years.

The Halifax process as described by Power (1962) and Idler (1967) essentially consists of two extractions with IPA containing close to 30% water, followed by a final extraction with anhydrous IPA. The purpose of the final extraction is to eliminate the residual fat and to dehydrate the proteinaceous residue. After the extraction the solvent usually is recovered as an azeotrope containing 12.3% water by weight or 9% by volume. The dehydration of an azeotrope is a costly process. The purpose of this study was to ascertain if the aqueous azeotrope could be successfully reused avoiding the process of its dehydration.

Materials and methods — Intensively feeding herring, in post-spawning stage, was caught off Halifax shores, immediately frozen at −40 C, and finely ground in an industrial meat chopper. After a thorough mixing, 100-g samples were weighed into extraction flasks. Calculated volume of IPA was added to each flask so that the moisture of the sample (67.2%) would make a mixture with the IPA containing 30% water. The total volume of liquid in each flask was adjusted to 300 ml by adding IPA containing 30% water. The samples were refluxed for 15 min, filtered while hot, washed twice with 25 ml of hot IPA containing the same amount of water as used in the extraction, and returned to the flasks for the next extractions. These were performed in the same manner using 150 ml of appropriate solvents. The filtrates were flash-evaporated and the crude extracts fractionated with ethyl ether into fat and so-called water-solubles. Both fractions as well as the filter cake were dried at 50–55 C under vacuum to a constant weight. The sequence of solvents and the results, which are averages of duplicates, are shown in Table 1.

Results and discussion — The survey of literature did not produce any information concerning the solubility of fish oils in dry or aqueous IPA. Some indications that the solubility is rather low, decreasing with dilution and increasing with temperature, can be taken from publications dealing with the solubilities of cottonseed oil in IPA–water mixtures (Harris et al., 1947, 1949; Harris and Hayward, 1950).

When fatty fish, such as herring, is used as a raw material, difficulties are encountered in producing a storage-stable FPC unless the residual lipid can be reduced to a very low limit. Power (1964) modified the Halifax process by substituting, in the second extraction, dry IPA for the aqueous solvent.

TABLE 1. Sequence of solvents used and results in percentages of weight of sample.

Solvents (IPA % v/v) Extractions			Fat				Water-solubles				Yield of FPC	Residual fat in FPC (%)
			Extractions				Extractions					
I	II	III	I	II	III	Total	I	II	III	Total		
70	70	99	11.9	1.9	2.3	16.1	2.1	0.4	0.1	2.6	15.0	0.69
70	70	Azeo	12.3	1.7	2.3	16.3	2.1	0.4	0.1	2.6	15.3	0.61
70	99	99	12.4	3.7	<0.1	16.1	2.4	0.1	<0.1	2.5	15.2	0.19
70	Azeo	Azeo	12.1	2.8	1.1	16.0	2.6	0.1	0.1	2.8	15.4	0.21
70	Azeo	99	12.2	2.7	1.3	16.2	2.3	0.1	0.1	2.5	15.6	0.23
Avg						16.2				2.6	15.3	

This allows production of FPC of very low fat content, but increases slightly the quantity of IPA used. In the Halifax process the raw material before extraction is acidified with polyphosphoric acid. In unpublished experiments performed in the Halifax Laboratory in 1954 it was found that the addition of any strong mineral acid to the raw material even in amounts not sufficient to bring the *p*H below 6.0 has a beneficial effect on the extraction of fat and water-solubles and efficiently neutralizes the odors. However, any addition of acid also increases the losses of protein into filtrates.

For better assessment of the efficiency of the azeotrope to defat and dehydrate the raw material, the use of acid in these experiments was omitted. For the same reason the volumes of solvent used in the second and third extractions as well as for the washings were kept to the minimum. The total fat present in the raw material was 16.25% as determined by exhaustive extractions using alternatively IPA with 30% water and anhydrous IPA. It can be seen from Table 1 that the first extraction removes up to 76% of the fat present in the sample whereas the second and the third on the average extract 16 and 9% respectively. The first extraction actually should remove identical amounts of fat from all samples. However, the variations are mainly due to the technical difficulties to maintain identical experimental conditions during the filtration. A slight decrease in the temperature during the filtration results in separation of fat from the mother liquor. This fat is reabsorbed and retained by the protein fibres. The deviations of the total fat values from the average value are well within the limits of experimental errors and cannot be solely attributed to the nature of the solvent. The variations in the residual fat in FPC are also insignificant. The residual fat was determined by a double soxhlet extraction — first with methanol then with chloroform and combining the extracts for evaporation. This illustrates that the azeotrope can be used in place of anhydrous IPA for production of FPC from herring.

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A Comparative Study of Purines Responsible for Silvering in Several Freshwater Fishes

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EALES, J. G. 1969. A comparative study of purines responsible for silvering in several freshwater fishes. J. Fish. Res. Bd. Canada 26: 1927–1931.

The major purines occurring in silvery depositions were analysed for several taxonomically diverse species of freshwater fishes by paper chromatography and ultraviolet-spectrophotometry.

Guanine and lesser quantities of hypoxanthine were the only purines found in the skin silvery layers of the channel catfish (*Ictalurus punctatus*), burbot (*Lota lota*), brook stickleback (*Culaea inconstans*), pike (*Esox lucius*), walleye (*Stizostedion vitreum*), sauger (*S. canadense*), goldfish (*Carassius auratus*), brook trout (*Salvelinus fontinalis*), and trout perch (*Percopsis omiscomaycus*). Possibly both guanine and hypoxanthine generally occur in fish skin silvery layers.

In the pike and walleye the purines in the homogenized eye were examined and guanine and hypoxanthine recovered. However, for the swimbladder of pike, walleye, and channel catfish guanine alone was found.

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GUANINE HAS BEEN REPORTED in the silvery layers of fish skin by Peschen (1939) and earlier workers (summarized by Peschen, 1939) and by Summer (1944), Hitchings and Falco (1944), and Robertson (1948). Zielgler-Gunder (1956) identified both guanine and hypoxanthine in the skin of the European chub (*Squalius cephalus*) and the bleak (*Alburnus lucidus*). More recently Markert and Vanstone (1966) found guanine, hypoxanthine, and possibly guanylic acid in the belly skin of parr and smolt coho salmon (*Oncorhynchus kisutch*); Johnston and Eales (1967) found guanine and appreciable amounts of hypoxanthine in both the skin and scale silvery layers in parr and smolt of Atlantic salmon (*Salmo salar*); and Hayashi and Saito (1968) found hypoxanthine and inosinic acid in the dermis of carp, rainbow trout, and chum salmon. Although it is generally assumed that the silvering of fish is due to reflection of light from guanine in a crystalline form, it has yet to be demonstrated that guanine is the only crystalline substance involved. Guanine may not be the only purine contributing towards silvering in teleosts. Furthermore, possibly both guanine and hypoxanthine occur generally in fish silvery layers but hypoxanthine was overlooked by earlier workers not using chromatographic and spectrophotometric techniques. Other purines could be present in some species of fish. Adenine, guanine, and hypoxanthine occur in the skins of several amphibia (Bagnara and Neidleman, 1958; Bagnara and Stackhouse, 1961; Stackhouse, 1966), and xanthine and uric acid deposits have been recorded

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in other animals (Fox and Vevers, 1960). In the present study the purines in the silvery layers of several taxonomically diverse freshwater fish species have been analyzed in the hope that some generalizations might emerge concerning the types and relative quantities of purines in fish silvery layers.

Qualitative methods — Methods were similar to those used by Johnston and Eales (1967). Purine compounds were extracted from tissues with adequate volumes of solvent (1 N HCl, 0.5 N NaOH, or 3 N NH_4OH), chromatographed on paper (descending) using several solvent systems (isopropanol–acetic acid–water (4:1:1 v/v) and methanol–HCl–water (7:2:1 v/v) were most commonly used), and the compounds located on paper by absorbence or fluorescence under ultraviolet (UV) (253.7 and 336.0 mμ). Tentative identification was made from sample and standard R_f values. Compounds were then eluted with the same solvent concentration used for initial extraction and the UV-absorption spectrum determined against an appropriate blank in a Unicam SP-800 recording spectrophotometer. Spectra obtained were compared with those of known substances subjected to a similar procedure.

Quantitative methods — Guanine and hypoxanthine levels were measured in several species for dorsal and ventral skin (including scales), swimbladder, and eye. Purines were extracted in 1 N HCl, chromatographed using methanol–HCl–H_2O (7:2:1 v/v), eluted in 1 N HCl and the concentration computed from the absorbence value relative to an appropriate blank on the spectrophotometer. Guanine and hypoxanthine levels were expressed as a percentage of the dry tissue weight (Johnston and Eales, 1967). For small fish, skin from the entire left dorsal (above lateral line) or entire left ventral (below lateral line) surface were examined. For fish exceeding 40 g, samples of dorsal or ventral skin from the mid-body region were used.

Since prolonged freezing slightly but significantly increases skin hypoxanthine levels of Atlantic salmon (C. E. Johnston and J. G. Eales, unpublished data), specimens frozen for longer than 1 week prior to analysis have been indicated.

Qualitative results — Specimens of channel catfish (*Ictalurus punctatus*), burbot (*Lota lota*), brook stickleback (*Culaea inconstans*), pike (*Esox lucius*), walleye (*Stizostedion vitreum*), sauger (*S. canadense*), goldfish (*Carassius auratus*), brook trout (*Salvelinus fontinalis*), and trout perch (*Percopsis omiscomaycus*) were examined. Both guanine and hypoxanthine were found in the skin of all species, although in some instances concentrations were very low. No other purines occurred in concentrations detectable on the chromatograms, although other UV-absorbing and UV-fluorescing compounds were observed. Most of these were probably pterins and no attempt was made to identify them. The possible presence of nucleosides and nucleotides was not explored intensively. However, none of eluates of the unidentified substances had UV-absorption peaks corresponding to those of commonly occurring nucleosides and nucleotides.

The purine contents of the silvery layer of the intact swimbladder of the pike, walleye, and channel catfish were examined. In each instance guanine alone was recovered.

The purine contents of the silvery layer in the eye were analysed in whole eye homogenates of the walleye and pike. Both guanine and hypoxanthine but no other purines were found. It is assumed that these purines were derived from the silvery reflecting layer (tapetum lucidum).

Quantitative results — The guanine and hypoxanthine levels for the dorsal and ventral surfaces were measured in two samples of yearling brook trout obtained from the Province of Manitoba hatchery at West Hawk Lake (Table 1).

TABLE 1. Mean guanine (% G) and mean hypoxanthine (% H) levels for the dorsal and ventral surfaces of yearling brook trout. The guanine/hypoxanthine (G/H) ratio is shown for each surface.

			Dorsal			Ventral		
Date	Wet wt of fish (g)	No. of fish sampled	% G	% H	G/H	% G	% H	G/H
May 1–15	13–21	14	1.90 (1.04)[a]	1.05 (0.26)	1.80 (0.34)	4.91 (1.23)	1.54 (0.27)	3.13 (0.39)
July 29–Aug. 6	10–34	10	1.66 (0.33)	0.86 (0.17)	1.95 (0.24)	5.90 (1.85)	1.85 (0.55)	3.19 (0.26)

[a]Standard error of the mean.

As expected guanine and hypoxanthine were more abundant ventrally. Most interesting is the significantly lower guanine/hypoxanthine (G/H) ratio for the dorsal surface where the hypoxanthine comprises a considerable proportion of the deposited purine.

The quantities of purines in dorsal and ventral skin, eye, and swimbladder were assessed for several other species (Table 2).

As in the brook trout purine levels were in all instances greater on the ventral surface. The absolute levels of purines varied considerably both between and within species. Comparison between tissues from different species is difficult because of the influence of skin thickness, and hence skin weight, on purine levels. However, comparisons of the G/H ratio can be made. As in the brook trout, the G/H ratio was generally greater ventrally. *Lota lota* was the only exception since it possessed no hypoxanthine dorsally and very low quantities ventrally. The G/H varied widely between species. Hypoxanthine was present in the eye but the G/H ratio was high.

Limited observations on nine species of teleosts from taxonomically diverse groups suggest that hypoxanthine as well as guanine may commonly occur in the skin silvery layers of teleosts. Although the ratio of guanine to hypoxanthine deposited varies greatly and may depend on several factors, it is usually lower on the dorsal surface. The G/H ratio was high for eye homogenates, but only guanine was deposited in the swimbladder. No other purine bases were detected in any of the tissues examined.

TABLE 2. Mean guanine (% G) and mean hypoxanthine (% H) levels for the dorsal (D) and ventral (V) skin, eye (E), and swimbladder (SB) of several species. The guanine/hypoxanthine (G/H) ratio is shown for each tissue.

Species	Source	Fresh wt of fish (g)	Date killed	Tissue	% G	% H	G/H
I. punctatus	Red R., St. Norbert, Man.	735	May 21	D	0.13	0.07	1.86
				V	0.54	0.09	6.00
				SB	1.27	0.00	–
	"	200	June 18	D	1.97	0.92	2.14
				V	2.99	0.58	5.16
L. lota	"	350	May 21	D	0.14	0.00	–
				V	2.35	0.06	39.2
C. inconstans[a]	Roseau R., Man.	1–2	June 29	D	0.69	0.57	1.46
				V			
E. lucius[b]	West Blue L., Man.	ca 1500	May 26	D	0.27	0.25	1.08
				V	1.71	0.76	2.25
				E	4.19	0.58	7.44
				SB	0.76	0.00	–
	Singush L., Man.	ca 1500	May 26	D	0.70	0.62	1.13
				V	4.85	1.26	3.85
				SB	1.22	0.00	–
S. vitreum[b]	West Blue L., Man.	ca 1200	May 26	D	0.08	0.05	1.60
				V	0.04	0.01	4.00
				E	4.20	0.41	10.24
				SB	0.46	0.00	–
	Red R., St. Norbert, Man.	600	June 18	D	0.27	0.25	1.08
				V	1.66	0.23	7.22
				SB	1.92	0.00	–

[a] Entire skins from seven individuals pooled; dorsal and ventral surfaces *not* separated.
[b] Specimens frozen from 1 to 6 weeks before analysis.

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Lobster Larvae off Pictou, Nova Scotia, Not Affected by Bleached Kraft Mill Effluent

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Scarratt, D. J. 1969. Lobster larvae off Pictou, Nova Scotia, not affected by bleached kraft mill effluent. J. Fish. Res. Bd. Canada 26: 1931–1934.

A survey made about 1 year after the opening of a bleached kraft mill near Pictou, Nova Scotia, indicates that the mill effluent is not having any immediate or direct effect on the distribution or abundance of lobster larvae.

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In September 1967, a 500 ton/day bleached kraft mill came into full production at Abercrombie Point, near Pictou, Nova Scotia. Freshwater effluent (BKME) is discharged continuously at a rate of ca. 20 million gal per day into a settling pond where it remains for 4 days before entering Boat Harbour, whence after a 40-day detention period it is discharged directly into the sea (Nova Scotia Water Resources Commission, personal communication).

Studies of the distribution of lobster larvae in late July and early August 1966 before completion of the plant (Scarratt, 1968) and of toxicity of BKME for lobster larvae (Sprague and McLeese, 1968a, b), and of the avoidance of reduced salinity by newly hatched lobster larvae (Scarratt and Raine,

1967) all suggested that the effluent would have no significant effect on the abundance and distribution of lobster larvae. The survey reported here was designed to test this hypothesis.

Lobster larvae were sampled from July 23 to August 5, 1968, using the same methods and equipment as in 1966. Seventeen of the 20 stations occupied in 1966 were reoccupied in 1968 (Fig. 1), with five 30-min surface plankton tows being made again at each station.

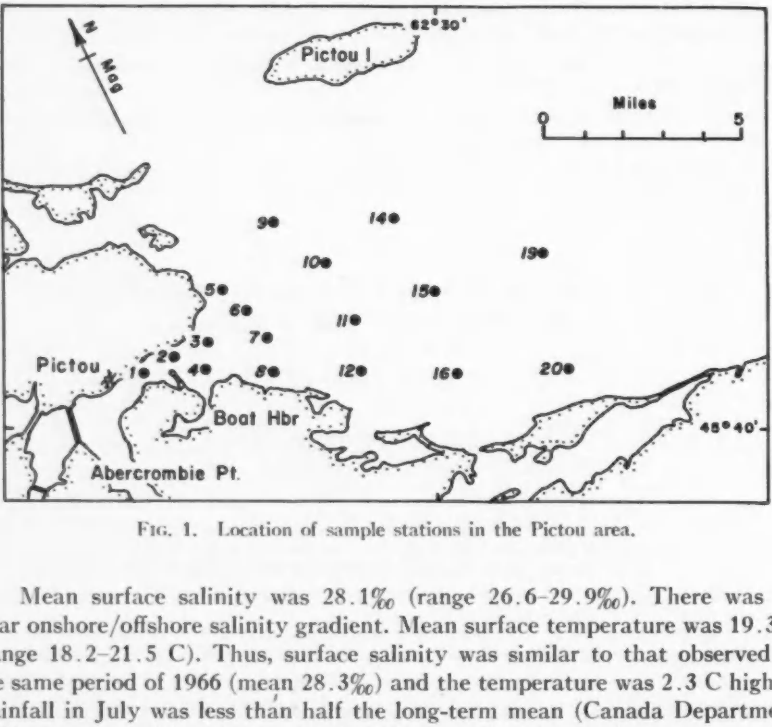

FIG. 1. Location of sample stations in the Pictou area.

Mean surface salinity was 28.1‰ (range 26.6–29.9‰). There was no clear onshore/offshore salinity gradient. Mean surface temperature was 19.3 C (range 18.2–21.5 C). Thus, surface salinity was similar to that observed in the same period of 1966 (mean 28.3‰) and the temperature was 2.3 C higher. Rainfall in July was less than half the long-term mean (Canada Department of Transport, 1968) and there were no heavy rains during the survey period. More onshore winds were recorded at sea than during the 1966 survey when the winds were predominantly southwesterly.

The higher water temperature in 1968 would result in more rapid larval development and the higher onshore wind component might result in a slightly closer onshore larval distribution. Neither is likely to affect the comparison between the two survey years.

A total of 746 larvae, comprising 673 in stage I, 15 in stage II, 3 in stage III, and 55 in stage IV, were taken at the 17 stations. The 1966 figures were a total of 1814, with 1721, 82, 6, and 5 for the four stages respectively at the same 17 stations. These differences in catches between the 2 years are well within the range of normal catch fluctuations found elsewhere in Northumberland Strait (Scarratt, 1964) and therefore provide no evidence for effects attributable to the pulp mill effluent. Effluent was detected by virtue of the colour and smell of the water at stations 1–4 in the mouth of Pictou Harbour and at station 8 a few miles to the east. An oceanographic survey, which included aerial mapping of the areas of discoloured water, made in September 1968 by Mr H. Neu and Dr D. Lawrence of the Atlantic Oceanographic Laboratory, Darmouth, N.S. (personal communication) confirms this general distribution. More larvae were taken within this area in 1968 than in 1966 (Table 1), including four in stage IV. This could be the result of the higher proportion of onshore winds in 1968. All the larvae taken were vigorous and appeared to be normal.

TABLE 1. Lobster larvae at same 17 stations, 1966, 1968, five tows per station.

Station	Stages I–III[a]		Stage IV	
	1966	1968	1966	1968
1[b]	0	0	0	0
2[b]	0	0	0	0
3[b]	8	0	0	1
4[b]	7	30	0	1
5	133	24	0	0
6	99	89	0	2
7	105	20	1	3
8[b]	4	23	1	2
9	87	56	0	1
10	347	46	1	5
11	156	44	0	6
12	13	145	0	4
14	88	80	0	2
15	168	59	0	5
16	124	19	0	3
19	253	40	2	5
20	217	16	0	15
	1809	691	5	55

[a]Stages I–III are grouped for convenience. These three stages comprise half of the time spent in the planktonic phase.

[b]Stations where, in 1968, surface water was at times discoloured by BKME.

There was no visual evidence of pulp mill effluent at any of the other stations where, in 1966 and again in 1968, most of the larvae were encountered.

It is clear from these results that the mill effluent at the time and place of sampling was not lethal to lobster larvae, and is not having any immediate, direct effect on the abundance and distribution of lobster larvae in the Pictou area.

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Occurrence of *Coregonus artedii* and *C. zenithicus* in Barrow Lake, Alberta

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PATERSON, COLIN G. 1969. Occurrence of *Coregonus artedii* and *C. zenithicus* in Barrow Lake, Alberta. J. Fish. Res. Bd. Canada 26: 1934–1939.

The variation in size at maturity and meristic characters of ciscoes inhabiting lakes in northern Alberta has made the assignment of specific names tenuous. Study of populations of *Coregonus artedii* and *C. zenithicus* in a small lake revealed that these two species can be effectively separated by gill-raker counts. A comparison with three other populations of *C. artedii* suggests that gill-raker counts will effectively distinguish these species in other northern Alberta lakes.

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A COLLECTION of ciscoes from Barrow Lake in northern Alberta appeared to contain two species which were easily differentiated on the basis of size. Fish belonging to the genus *Coregonus* occur in many lakes in the northern half of Alberta but the great variation shown in the number of lateral line scales and gill rakers, as well as the size reached at maturity, has made it difficult to assign specific names to many populations. The occurrence of two sympatric species in a small lake offers the opportunity to compare two species under similar environmental conditions. It is then hoped that the distinguishing characters may be applied to other populations within the province.

The ciscoes of Alberta — Dymond and Pritchard (1930) list *Coregonus artedii* Lesueur, *C. nigripinnis* (Gill), and *C. zenithicus* (Jordan and Evermann) from Lake Athabaska. Rawson (1949) records *C. zenithicus* and *C. artedii* from Lake Athabaska but does not include *C. nigripinnis*. Scott (1958) does

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not include *C. zenithicus* in the Alberta fauna but if it is present in Lake Athabaska in Saskatchewan then it most probably extends into the Alberta portion of the lake. The small amount of published material on ciscoes in other Alberta lakes (Miller, 1950; Dymond and Pritchard, 1930) refers only to *C. artedii.*

Barrow Lake — Barrow Lake is a small lake of 1.47 sq miles lying in the MacKenzie lowlands at an altitude of 650 ft. It lies at 59°15′N, 111°14′ W, approximately 35 miles north of Ft. Chipewyan, Alberta. The lake drains via a small creek into the Slave River. Barrow Lake was visited during the period August 8–11 and again August 17–20, 1966. At this time the water temperature varied from 14.3 C at the surface to 5.2 C at 42 ft. Below this depth there was a temperature inversion so that a temperature of 6.3 C was recorded at the maximum depth of 60 ft. The oxygen concentration was 10 ppm at the surface and 3 ppm at a depth of 60 ft.

Materials and methods — Fish were collected with nylon gillnets with a depth of 5 ft which were fished as sets of 100 yards of 4-inch mesh and as a gang composed of 50-yard lengths of 1½, 2½, 3½, 4½, and 5½ inch stretched mesh. When it was found that the smaller form of cisco (*C. artedii*) was captured only in the 1½ inch mesh and only in the deeper water, this net was removed from the gang and fished independently. All nets were in the water for six nights and were checked daily. Seining with a 30-ft seine of 4 inch mesh failed to produce any ciscoes.

Only 16 of the larger form (*C. zenithicus*) were captured. These were all taken in the 3½- and 4-inch mesh nets set in 6–15 ft of water. No specimens were obtained in deeper water although these mesh sizes were often fished at depths dôwn to 60 ft. *Coregonus artedii*, in contrast, was only taken in 1½ inch mesh and only in sets made in more than 45 ft of water. Approximately 190 of this smaller form were captured.

Fork length, weight, and state of maturity were determined in the field for all *C. zenithicus* and for a random sample of 30 *C. artedii.* Four specimens of the larger form had been severely damaged by northern pike (*Esox lucius*) while in the nets so that only the first gill arch on the left side was removed from three of these specimens and preserved intact in 10% formalin.

Counts of gill rakers were performed on the excised first left arch for 21 specimens of *C. artedii* and 15 *C. zenithicus.* Counts were made using a binocular microscope and all bony rudiments were included. Lateral line scales included all pored scales and in the case of *C. artedii* these counts were also done with the aid of magnification. Several body measurements were determined after the methods of Hubbs and Lagler (1958) using dial calipers. These body proportions were determined for the 12 intact specimens of *C. zenithicus* and for 10 *C. artedii* picked at random from the specimens used for gill-raker and lateral line scale counts.

Results — Although it was difficult to find differences in body proportions between the two species occurring in Barrow Lake, it was a simple matter to differentiate the two forms on the basis of size. With the possible exception of one specimen all those less than 21 cm fork length were referrable to *Coregonus artedii* as described by Dymond and Pritchard (1930) and Koelz (1929). Fish of this species ranged from 16.8 to 20.8 cm fork length and weighed from 30 to 130 g. The age of 10 specimens encompassing the length range was from 2 to 4 years. The sex ratio was 24 males:20 females. The larger form occurring in the lake most closely resembles *C. zenithicus* and was captured between 27.7 and 46.7 cm fork length and a weight of 340 to 1680 g. The age range of these specimens was from 5 to 11 years. The sex ratio of 15 specimens was 7 males:8 females.

Table 1 summarizes certain of the body proportions determined for the two species. The great amount of overlap in the relative body proportions of the two species makes these characters of little use in separating the two forms. The only ratios which were found to be significantly different between the two species were the ratio of fork length divided by snout to dorsal origin and head length divided by snout to orbit distance. These characters were significantly different at the 95 and 99% level respectively.

TABLE 1. Body proportions of the two forms of *Coregonus* occurring in Barrow Lake, Alberta.

	C. artedii		*C. zenithicus*	
	Avg	Range	Avg	Range
Fork length (*cm*)	18.5	(16.8–20.8)	37.6	(27.7–46.7)
$\frac{\text{Fork length}}{\text{Head length}}$	4.49	(4.25–4.67)	4.34	(4.04–4.54)
$\frac{\text{Fork length}}{\text{Caudal peduncle depth}}$	12.11	(11.4–12.7)	12.32	(11.5–13.1)
$\frac{\text{Head length}}{\text{Interorbital width}}$	3.90	(3.53–4.33)	4.03	(3.68–4.60)
$\frac{\text{Fork length}}{\text{Snout to dorsal origin}}$	2.28	(2.21–2.41)	2.14	(2.10–2.24)
$\frac{\text{Maxilla length}}{\text{Interorbital width}}$	1.39	(1.26–1.67)	1.53	(1.37–1.70)
$\frac{\text{Head length}}{\text{Maxilla length}}$	2.81	(2.59–2.99)	2.64	(2.40–2.81)
$\frac{\text{Maxilla length}}{\text{Maxilla width (max)}}$	3.7	(3.1–4.1)	3.88	(2.05–5.00)
$\frac{\text{Dorsal fin height}}{\text{Dorsal fin base}}$	1.77	(1.61–2.00)	1.59	(1.04–1.96)
$\frac{\text{Fork length}}{\text{Body depth (max)}}$	4.12	(3.28–4.50)	3.76	(3.53–3.95)
$\frac{\text{Snout to adipose origin}}{\text{Snout to dorsal origin}}$	1.73	(1.65–1.80)	1.65	(1.57–1.71)
$\frac{\text{Head length}}{\text{Snout to orbit}}$	3.99	(3.75–4.20)	3.53	(3.24–3.80)
$\frac{\text{Fork length}}{\text{Snout to anal origin}}$	1.42	(1.32–1.49)	1.38	(1.34–1.42)

The number of gill rakers is the major character used by Dymond and Pritchard (1930) to separate the ciscoes found in western Canada. As shown in Table 2, the separation of the two species in Barrow Lake is complete on

TABLE 2. Number of gill rakers and lateral line scales of the two forms of *Coregonus* occurring in Barrow Lake, Alberta.

	No. of gill rakers on first arch															
	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	Avg
Coregonus artedii								1	1	2	5	7		3	2	47.8
C. zenithicus	1	1	6	3	4											39.5

	No. of lateral line scales														
	64	65	66	67	68	69	70	71	72	73	74	75	76	77	Avg
C. artedii	1	3	4	1	3	1	3	2	2		2				68.5
C. zenithicus						1	2	1	1	3	1		2	1	72.8

the basis of the number of gill rakers, *C. artedii* having more than 43 and *C. zenithicus* always less than 42. Table 2 reveals that the number of lateral line scales tends to be greater in *C. zenithicus* than in *C. artedii* but the amount of overlap is so great that this character is only of limited use in delineating the two species.

It may be argued that the two size classes in Barrow Lake represent different age classes of the same species as the smaller form had an age range of 2–4 years whereas the larger specimens ranged from 5 to 11 years. This argument would also be substantiated by the fact that many of the specimens of the larger form have a greater size than the maximum recorded for *C. zenithicus* by Scott (1954) and Koelz (1929). However, the wide separation on the basis of the number of gill rakers would suggest the presence of two species.

The author has examined three small collections of ciscoes from Myer's Lake, Lac la Biche, and Wolf Lake, Alberta. These fish closely resemble the size normally taken by commercial fishermen in Alberta with the Myer's Lake sample ranging from 15.5 to 29.5 cm fork length and the Lac la Biche fish ranging from 21.5 to 30.5 cm fork length. In a sample of 10 fish from Myer's Lake the number of gill rakers varied from 46 to 50 with an average of 47.8. Nine specimens from Lac la Biche had a range of gill-raker number from 42 to 49 with an average of 45.2. Gill-raker counts for 36 ciscoes from Wolf Lake gave a range of 45–54 with a mean of 49.0. It would therefore appear that these three populations may be referred to *C. artedii.*

The two species of cisco known to occur in the smaller lakes of Alberta, namely *C. artedii* and *C. zenithicus*, can best be separated on the basis of the number of gill rakers on the first arch; *C. artedii* have 42 or more and *C. zenithicus* have less than this number. If *C. nigripinnis* is found to occur in Alberta, other than in Lake Athabasca, it can be separated from *C. artedii* on the basis of its longer head. The ratio of body length to head length is stated to be more than 4.0 in *C. artedii* and less than 4.0 in *C. nigripinnis* (Dymond and Pritchard, 1930). *Coregonus nigripinnis* usually possesses more than 42 gill rakers on the first arch which serves to distinguish this species from *C. zenithicus.*

Three of the specimens of *C. zenithicus* and six *C. artedii* have been deposited in the Royal Ontario Museum Acc. No. 1485.

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Uptake of Dieldrin by Isolated Perfused Gills of Rainbow Trout

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FROMM, PAUL O., AND ROBERT C. HUNTER. 1969. Uptake of dieldrin by isolated perfused gills of rainbow trout. J. Fish. Res. Bd. Canada 26: 1939–1942.

Transfer of dieldrin from environmental water into the vascular system of isolated perfused gills of rainbow trout was demonstrated. Transfer occurred only when plasma protein, or more probably plasma lipoprotein, was present in the perfusion fluid. Over the range of concentrations tested transfer was always down a concentration gradient. Larger quantities of dieldrin were absorbed on or bound in gills perfused with plasma than in those perfused with Ringer solution or a protein-free synthetic tissue culture medium (TC-199).

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DIELDRIN, a cyclodiene, is a widely used pesticide with a relatively long residual life in the environment. Aquatic organisms such as fish may assimilate dieldrin from water through the gill membranes or skin or via the alimentary tract. Holden (1962, 1965), Premdas and Anderson (1963), Crosby and Tucker (1966), and Lenon (MS, 1968) have all suggested that uptake of DDT and related insecticides including dieldrin occurs primarily by way of the gills. No specific data on gill uptake were presented by these workers but the extremely rapid uptake and dispersal of DDT in fish led them to conclude that the gills are the chief port of entry and the blood is the chief means of transport of assimilated insecticides.

The purpose of our experiments was to investigate the transfer of dieldrin from the environmental water into the vascular system of isolated perfused gills of rainbow trout.

Materials and methods — Rainbow trout (*Salmo gairdneri*) weighing from 125 to 410 g were killed by bleeding, and gill arches were surgically removed and attached to a gill-perfusion apparatus as described by Richards and Fromm (1969). The arches were perfused initially with Ringer solution containing glucose for about 15 min to clear the filaments of blood cells. During this time the gills were bathed with the nutrient Ringer solution.

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Dieldrin was dissolved in ethanol (10 mg/ml) and bath solutions containing approximately 1000 ppb (parts per billion or μg/L) were prepared by adding 0.1 ml of dieldrin stock solution to 1 liter of dechlorinated tap water. Bath solutions were prepared at least 24 hr prior to use. About 80 ml were then put into a glass beaker and stirred continuously with a teflon-coated stirring bar. Analyses of samples indicated that during the 80-min equilibration period a variable amount of dieldrin was volatilized or adsorbed on the beaker walls so that the initial concentrations to which the gill arches were exposed varied from one experiment to another. All experiments were performed in a walk-in refrigerator at 13 C.

To closely mimic normal physiological conditions the initial perfusion fluid used was heparinized plasma. Since it was difficult to obtain sufficient volumes of plasma from a single fish for perfusion and we disliked the idea of using pooled samples from several donor fish, a fish Ringer solution containing glucose was substituted as the perfusion fluid. When no dieldrin appeared in the perfusate of Ringer perfused gills we used TC-199 instead of the Ringer solution. The synthetic tissue culture medium (TC-199) is a product of Difco Co., Detroit, Michigan, which contains salts, vitamins, and several amino acids but no protein.

In a typical experiment a 1-ml aliquot of the dieldrin bath was taken for analysis at the time the gills were placed in the solution and a second aliquot was taken after 1 ml of perfusate had been collected from the gill. A 1-ml aliquot of the perfusion fluid was also taken for analysis. For determinations of the amount of dieldrin adsorbed on or bound to gill tissues, gills were removed from the bath, flushed with Ringer solution for 3–5 min, weighed, and then digested with 20% alcoholic KOH for 1 hr at 76 C.

For analytical consistency all solutions analyzed were subjected to the same digestion procedure as the gill tissues even though not all of the samples contained protein material. After digestion all samples were individually extracted with petroleum ether from which water was removed using anhydrous Na_2SO_4. The dieldrin content of the final solution was determined by gas chromatography. In our initial analyses ether extracts were run through a Florisil column to eliminate co-extractives, and many of the smaller peaks in the chromatogram were eliminated. This procedure was deemed unnecessary and was discarded in the studies reported here. The chromatograph used was an Aerograph HY-FL model 600-C (Wilkens Inst. & Res. Inc.) with a proportional isothermal temperature control (Model 328) and an electron-capture detector. The 5′ × ⅛″ pyrex glass column was packed with 3% QF-1 on 100/120 mesh Gas-Chrom Q. Column temperature was 180 C and gas (nitrogen) flow 40 ml/min.

Results — Typical gas chromatography tracings of the solutions analyzed in the experiments (Fig. 1) show that no change occurred either in Ringer solution (experiment 1A, B) or in TC-199 (experiment 2A, B) during passage through isolated gills. That none of the peaks which appear in the tracings

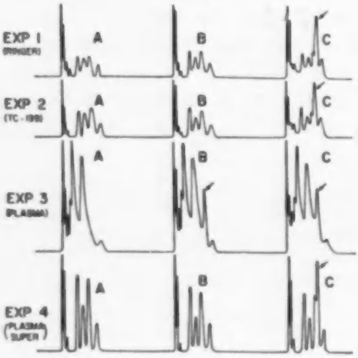

FIG. 1. Gas chromatography tracings of (A) perfusion fluid, (B) perfusate, and (C) perfusate to which a known amount of dieldrin was added (spiked), excepting that experiment 3C is spiked perfusion fluid. In experiments 1, 2, and 3 the gills were perfused with Ringer solution, TC-199, and rainbow trout plasma respectively. Tracings for experiment 4A and B are of the supernatant solutions which remained after plasma samples had been treated with barium hydroxide–zinc sulfate and centrifuged to remove protein. Experiment 4C is a chromatogram for spiked supernatant solution. Dieldrin peaks are indicated with solid black arrows.

of the experimental solutions represented dieldrin is shown by tracings of spiked samples (experiment 1C and 2C). When isolated gills perfused with plasma were placed in a bath of tap water containing dieldrin the insecticide was found in the plasma perfusate (experiment 3B). Tracings of plasma perfusate from gills which were not exposed to dieldrin were identical to those for nonperfused plasma (experiment 3A), whereas samples of spiked nonperfused plasma (experiment 3C) were identical to those for plasma from dieldrin-exposed gills (experiment 3B). Although the tracings are not shown, spiking of plasma from dieldrin-exposed gills caused an increase in size or height of the dieldrin peak and confirmed the original presence of dieldrin in these samples.

The proteins in two samples of plasma perfusate from gills exposed to dieldrin and in a sample of nonperfused plasma were precipitated using barium hydroxide and zinc sulfate (Somogyi procedure). Gas chromatographic tracings of the supernatant fluid from these samples (Fig. 1, experiment 4A, B) indicate that the dieldrin present in the plasma samples was precipitated along with the plasma proteins.

Large amounts of dieldrin were found adsorbed on or bound to perfused gills (Table 1) with relatively greater quantities being found associated with plasma-perfused gills than those perfused with Ringer solution. The dieldrin concentration in the plasma perfusate was uniformly less than that in the solution which bathed the gills (Table 1) and over the range of concentrations tested the amount of dieldrin transferred across isolated gills appeared to be dependent upon the concentration of the insecticide in the bath.

TABLE 1. Summary of information on amount of dieldrin (ppb) bound to perfused gills and effect of concentration of dieldrin in bath on dieldrin transfer by gills.

Perfusion fluid	No. of perfusions	Concn of dieldrin in bath	Dieldrin bound to gill	Concn of dieldrin in perfusate
Ringer	2	None	No	None
Ringer	8	119–339	Yes[a]	"
TC-199	2	None	—[b]	"
TC-199	2	289	—[b]	"
Plasma	2	None	No	"
Plasma	9	169–515	Yes	64–220

[a] But much less than that found in plasma perfused gills.

[b] Not analyzed for dieldrin.

Discussion — We have clearly demonstrated that dieldrin can be transferred from environmental water into the vascular system of isolated perfused gills of rainbow trout. This transfer occurred only when protein, or more probably lipoprotein, was present in the perfusion fluid. Since the dieldrin concentration in the plasma perfusate from gills was always less than that of the bath fluid a gradient for inward diffusion existed and there is no need to postulate any other mechanism for inward movement of the insecticide by the gill. The greater quantities of dieldrin found in gills perfused with plasma than in those perfused with Ringer solution may indicate either a greater entry of dieldrin into epithelial cells of gills perfused with plasma and a consequent greater internal binding of the insecticide or inadequate flushing of the gill lamellae prior to analyses. As the plasma used had been obtained by bleeding the fish from which the gills were taken, it is probable that epinephrine was released into the blood during the blood letting procedure. Epinephrine causes high blood flow into lamellae of perfused gills (Richards and Fromm, 1969) and it may be that the lamellae were not flushed out by the Ringer solution, which contained no epinephrine.

Lipoprotein is present in mammalian blood and evidence has been accumulating that much of the lipid material in human plasma is combined with protein. This may also be true for fish plasma. We suggest that dieldrin and other related insecticides diffuse through gills of fishes and are dissolved in the lipid portion of plasma lipoprotein, in which form they are transported to and become dissolved primarily in the lipid portion of the various tissues. Since the insecticide is much more soluble in lipid than in water the tissue concentrations can attain levels far above that of the environmental water independent of any active transport mechanism. Little is known about the pathways used for the excretion of dieldrin but since release is rapid (Gakstatter and Weiss, 1967; Lenon, MS, 1968) it appears unlikely that excretion of most of the dieldrin is dependent upon lipid turnover in the tissues.

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Aspects of the Anatomy of the Winter Flounder Ovary with Hypotheses on Oocyte Maturation Time

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Ovarian tissues of mature winter flounders have distinct groups of oocyte types which suggests that a minimum of 2 or 3 years pass from the time that oocytes become histologically recognizable until they are spawned.

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LITTLE GROSS ANATOMY and no microanatomy of the ovary of the winter flounder (*Pseudopleuronectes americanus*) has been published. The anatomical information considered here places the winter flounder among those northern marine fishes which take 2–3 years for oocyte development. The data were obtained during an experiment designed to test the effect of food supply on aspects of the physiology of the winter flounder (Dunn, MS, 1967).

The left ovary of 103 fish 35–45 cm long was fixed in 5% formalin, imbedded in paraffin, stained with Harris' hematoxylin and Gomori's trichrome, and sectioned transversely at 7 μ. Sections were made at a plane spaced one-third the length of the ovary as measured from the anterior end. A few sections were also made at two-thirds the length of the ovary measured from the anterior end.

The proportions of oocyte types in ovaries were determined by counting all oocytes sectioned through the nucleus in an optical field. Enough adjacent fields were taken to complete an "X" pattern centered on each ovary section and running to the ovary wall. No differences were found in the proportions of oocyte stages at the two sectioning positions, and so the smaller posterior section was dropped from the study.

Oocyte diameters were sampled by measuring the first 100 oocytes encountered while determining proportions of oocyte types. Only oocytes sectioned through the nucleus were measured. No attempt was made to identify or measure cells less than 10 μ in diameter.

The winter flounder ovary is a modification of the vertebrate compact ovary (Nelson, 1953), being adapted to the flounder's exceptional physical

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form. A photograph is given by Topp (1968). The ovaries are gently tapered posteriorly, but anteriorly they are dorso-ventrally broadened and abruptly truncated where they meet the first loop of the intestine. Though most teleost ovaries have a hilus and mesovarium running the length of the organ, the hilus is relatively short in winter flounder, the ovaries being connected anteriorly to the peritoneum by a short mesovarium. Ovarian arteries and veins pass through the hilus.

The ovigerous lamellae run longitudinally, branching dichotomously within the ovary. The lamellae arise from the proximal wall of the ovary. Internal to the distal side of the ovary is the ovarian sinus which continues anteriorly to join the short oviduct.

Four oocyte types were distinguished morphologically, and are as follows:

(*a*) *Small immature oocytes* — These ranged from 10 to 80 μ in diameter and were irregular in shape, sometimes sharply angled. They were darkly staining oocytes (basophilic) containing no yolk or fat droplets. The nucleus of each contained one or two large, irregularly placed nucleoli. Threads of chromatin are evident in the nucleoplasm. This oocyte stage seems identical to the early perinucleolus stage described and photographed by Yamamoto (1956) for the flounder *Liopsetta obscura*.

(*b*) *Large immature oocytes* — These were round, yolkless, and 80–150 μ in diameter. They contained some fat droplets which gave a granular appearance to the cytoplasm. The nucleus of each contained several darkly staining nucleoli arranged around its periphery with threads of chromatin material evident in the nucleoplasm. These oocytes seem to be the same as the late perinucleolus stage of Yamamoto (1956).

(*c*) *Maturing oocytes* — These were oocytes which had begun vitellogenesis. They were characterized in all but the earliest forms by a massive deutoplasm (mostly fat and yolk deposits), a small protoplasm, and prominent thecae. The first visible yolk deposition occurred in the immature oocytes when they were about 150 μ in diameter. At ovulation, mature oocytes are 740–850 μ in diameter (Bigelow and Schroeder, 1953). Yamamoto (1956) described maturing oocytes in *Liopsetta* throughout their developmental period and classified seven substages. These substages likely would be found in winter flounder if a similar study were made.

(*d*) *Atretic or regressing oocytes* — Some characteristics associated with oocyte atresia in winter flounder are: shrinkage of the zona radiata away from the theca, disorganization of cellular structure including rupture of the nucleus and pronounced wrinkling of the theca, continuous resorption of yolk and fat, which, when complete, leaves a mass of folded theca often containing a darkly staining amorphous material. Photomicrographs of regressing follicles may be found in Bowers and Holliday (1961) and Wiebe (1968).

Three states of ovary development were distinguished for fish held in tanks on the basis of histological examination of follicular development.

(*a*) *Resting ovaries* — These contained no oocytes with yolk deposits. The ovaries were usually characterized by large intra-ovarian spaces and relatively thick walls. Some of them, however, were similar to ovaries of immature fish with relatively small intra-ovarian spaces and thin walls. It would be surprising to find that the large flounders (greater than 34 cm) used in this study had not spawned at least once (McCracken, MS, 1954). Resting ovaries were usually from fish held on reduced rations.

(*b*) ***Ripening ovaries*** — These contained maturing oocytes as well as atretic and immature oocytes.

(*c*) *Regressing ovaries* — There were ovaries where a portion of the large immature oocytes had begun vitellogenesis normally, but at the time of sampling were all atretic. These ovaries were usually less emaciated than the resting type, and the immature oocytes more densely arranged. This ovary condition probably resulted from experimental conditions.

Resting stage ovaries were examined to test whether there was a continuous size gradation between the small immature oocytes and large immature oocytes described morphologically in the above paragraphs. Of the 103 fish held in tanks for periods of 3–6 months, 28 had resting ovaries. It was considered that these ovaries had recuperated from the previous spawning (late April and May (McCracken, MS, 1954))but had not lost immature oocytes to the maturing and atretic stages as had ripening and regressing ovaries. They should yield information on frequencies of oocyte types for the period of summer before onset of vitellogenesis. Effect of ration level on oocyte frequencies in ripening and regressing ovaries will be dealt with in another paper.

Frequency distributions of oocyte sizes in resting ovaries for various sampling dates were compared first. No growth in oocyte diameter was apparent over the sampling period (September–December 1966). The frequency distributions from the 28 ovaries were nearly identical and so the frequency distributions were combined (Fig. 1). There was clearly not a continuous size gradation of oocyte diameters, but a bi-modal distribution. The small immature oocytes (as distinguished structurally) were associated with the 40- to 50-μ mode whereas the large immature oocytes were associated with the 100- to 110-μ mode.

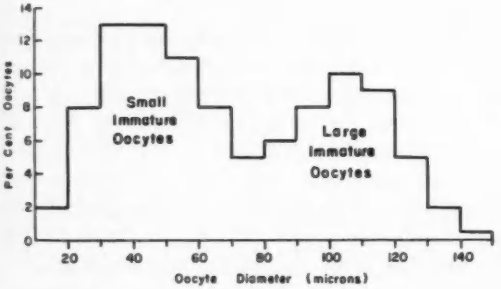

FIG. 1. Frequency distribution of oocyte diameters from resting stage ovaries.

The mean percentages of small immature oocytes and large immature oocytes in resting ovaries were determined on the basis of oocyte morphology.

The mean frequency of the large immature oocytes was 33.6% (standard deviation = 3%). The small immature stage made up the other 66.4% of the oocytes (SD = 3%).

In a sample of 14 flounders with ripening ovaries that were fixed in formalin immediately after capture from Passamaquoddy Bay in early September 1966, small immature oocytes averaged 63% (SD = 5%) of all the oocytes; large immature oocytes comprised 4% (SD = 3%); maturing oocytes, 29% (SD = 3%); and atretic oocytes, 4% (SD = 3%).

Prior to the onset of vitellogenesis in late August then, the immature oocytes in winter flounder ovaries had separated into two recognizable stocks. We suggest that only the large immature oocytes of the resting ovaries would have developed yolk during the year that the ovaries were sampled since the Bay fish in September still retained 63% of their oocytes in the small immature stage but only 4% in the large immature stage. The other large immature oocytes had apparently progressed to maturing or atretic stages.

One might set up a null hypothesis that the resting ovaries examined in the fall of 1966 would contain equal numbers of small and large immature oocytes instead of the one-third to two-third ratio found, since, at first glance, the large immature oocytes would seem to represent the 1967 spawning and the small immature oocytes the 1968 spawning. Three alternatives seem to be open to account for the empirical departure from the null hypothesis. Assuming that all the small immature oocytes were produced in 1966 and that the smaller number of large immature oocytes were produced in 1965, then either 1965 was not so conducive to oocyte formation as was 1966, or, secondly, some 50% of the oocytes that were produced in 1965 disappeared in atresia. Thirdly, all of the small immature oocytes might not have been formed in 1966. One-third of them might have been produced in 1966, and a third in 1965. In this instance, the large immature oocytes would likely have been produced in 1964.

Possibly, then, there is either a 2- or 3-year period between the time that the oocytes become histologically recognizable and their discharge as mature ova. One hypothesis for the winter flounder would be that, at the onset of year I, the small immature oocytes become recognizable; during year II, these oocytes become large immature oocytes; and during year III, yolk is deposited in the oocytes and they are spawned. Implicit in this hypothesis is the assumption that the youngest group of small immature oocytes becomes differentiated before the year-old small immatures develop to the large immature stage, since, in the Bay fish, the large immatures had progressed to the mature stage whereas 63% of the oocytes (possibly two groups each comprising one-third of the oocytes) remained as small immatures.

The alternate hypothesis is that the oocytes become recognizable and develop to the large immature stage during the 1st year, and during the 2nd year undergo vitellogenesis and ovulation. In view of the coincidence that one-third of the oocytes are at the large immature stage and two-thirds at the small immature stage, we currently favour the hypothesis of a 3-year minimum development period. Furthermore, acceptance of a 2-year hypothesis would also mean acceptance of the condition that fecundity can vary from year to year by at least 50%, which seems extreme.

There is good evidence of long developmental periods for oocytes in other northern demersal fishes. Yamamoto (1956) suggested a 2- or 3-year period of oocyte development for the Pacific flounder, *Liopsetta obscura*, on the basis of oocyte morphology. Two-year minimum periods for oocyte development have been suggested for the European plaice *Pleuronectes platessa* (Franz, 1910), the haddock *Melanogrammus aeglefinus* (Raitt, 1933), and the hake *Merluccius merluccius* (Hickling, 1930).

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Two Useful Devices for Vertical Plankton and Water Sampling

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Two devices for taking plankton and water samples are described. One, an inexpensive syringe sampler, will obtain small uncontaminated samples from thin strata in lakes. The other, a self-activating transparent zooplankton trap, combines many of the advantages of both net and trap-type samplers. It is capable of removing the smallest zooplankton and its light weight allows large samples to be taken easily from a small boat. Comparisons with other samplers prove the self-activating trap to be more efficient than other devices which are commonly used.

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In the course of investigating the plankton and water chemistry of several small lakes in the Canadian Shield it was necessary, both from a standpoint of cost and of function, to develop two new sampling devices rather than use those commonly employed in limnological investigations. Both devices are described here, in hope that they may prove useful to other investigators facing similar sampling problems.

Device 1. The first device is one designed for obtaining samples from thin undisturbed strata within a water column. The sampler was designed as a means for sampling near the thermocline, chemocline, or bottom of a lake without the disturbance associated with most commonly used sampling devices. The sampler may be constructed in most laboratory workshops for a cost of $15–20.

Sampler construction — The body of the sampler is an inexpensive disposable plastic syringe of 50 ml capacity. Such syringes are made completely of inert material, and come in sizes ranging from 1 to 1000 ml, so that with appropriate modifications several sizes of sampler might be constructed. A single syringe will normally last several seasons, and may be replaced in a matter of minutes with pliers and screwdriver. A bayonet-style mount allowing syringes to be changed more rapidly could easily be constructed.

All other parts of the sampler are constructed of brass, with the exception of the three slide-rods which are of stainless steel. Construction detail is shown in Fig. 1.

Sampler operation — The sampler is lowered in the closed position, as shown in Fig. 2A. Dropping a messenger-weight releases catch B (see Fig. 1) allowing the weighted barrel of the syringe to move downward until it comes

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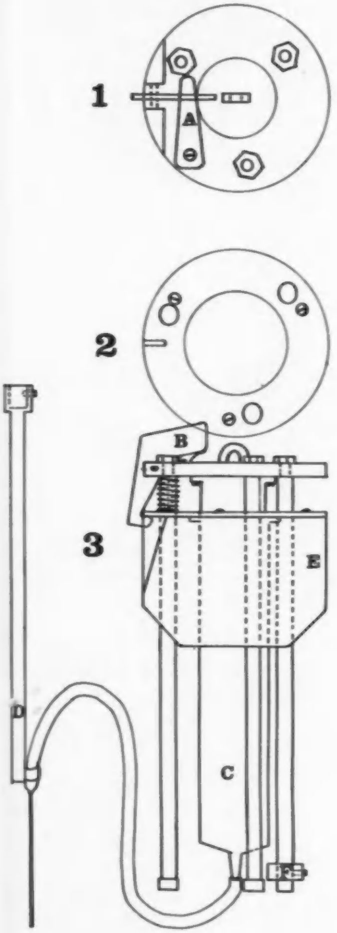

FIG. 1. Construction details for the syringe sampler. (1) Top view; (2) top view of weight; (3) side view. See text for explanation.

against stop F, drawing water into the barrel of the syringe. Stop F is movable, so that the volume drawn by the sampler may be adjusted. The sampler in filled position is shown in Fig. 2B.

In its simplest form the sampler may be used without the syringe needle attachment. The aperture of the 50-ml syringe moves a maximum of 12 cm vertically while filling. To draw samples from very thin layers where turbulence must be completely avoided, the syringe-needle attachment shown in Fig. 1 and 2 should be used. This consists of an extension rod to which a large bore syringe needle may be attached by a clip. The needle is connected to the aperture of the syringe barrel by a length of flexible tygon tubing.

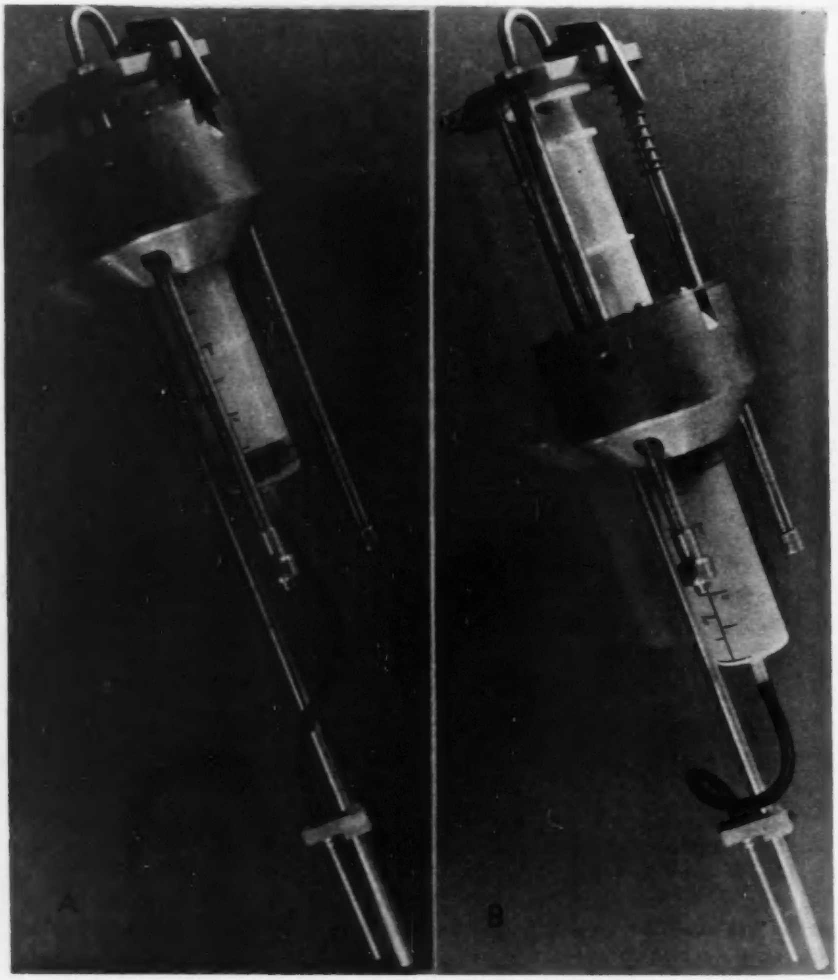

FIG. 2. (A) Syringe sampler in empty position; (B) syringe sampler in full position.

If lowered slowly into position, disturbance of strata by the sampler should be very slight.

Microbiological sampling — If sterile samples are desired, a separate length of tubing, complete with a syringe needle and a small plug of cotton in the upper end of the tubing, should be prepared for each sample to be taken. Each needle and tube should be sealed in a separate container for autoclaving. As the needle is held by a simple clip, tubes can be speedily changed between samples. The volume held by the sterile tube is sufficient to inoculate several cultures.

Device 2. The second device is a modification of Patalas' self-activating plankton trap. All popular zooplankton samplers have several undesirable characteristics. Most of the metered netting devices, including the frequently used Clarke–Bumpus sampler, are priced beyond the budget of a private investigator or a small laboratory. In addition, a power boat is usually required for towing except when vertical tows are satisfactory. The metering devices commonly employed will not operate properly when fine mesh townets are used; therefore, quantitative samples of small organisms cannot be obtained. Complicated opening and closing devices are necessary if metered nets are to be used for obtaining vertical distribution data, and the depth of the sampler must be calculated from the length and angle of the tow wire. There is recent evidence to suggest that tow bridles of any sort have a detrimental effect on the efficiency of nets (Mahnken, 1967). Avoidance reactions, particularly by larger zooplankton are also of importance, as discussed later.

Trap-type samplers, including the designs of Juday (1916), Rawson (1956), and Patalas (1954), as well as the large van Dorn or Kemmerer samplers, suffer from one or more of the following objections:

1. Many samplers have complicated tripping mechanisms which do not function dependably in cold weather, are difficult to reset, and require a messenger for closing. Taking several samples is a tedious process.

2. The sample size is too small, or, if a larger sampler is used, it is heavy and difficult to lift into a boat when full of water. Most must be held over a net for emptying.

3. Most have been constructed of opaque materials which may cause avoidance reactions from some planktonic organisms (see Szlauer, 1964).

The sampler described here is in many respects similar to that of Patalas (1954). It is, however, almost entirely constructed of transparent material, and its light weight and method of emptying make it possible to use samplers of up to 100 liters from even a small car-top boat.

A picture of the sampler in operation is given in Fig. 3, and construction details for a model of 28.7 liters capacity are given in Fig. 4. The body of the sampler is entirely constructed of plexiglass sheeting.[1] This material has proven extremely durable during one season's field work. Small repairs may be quickly made in the field using chloroform as a solvent. A small nannoplankton net is attached to the side ring for filtering the contents of the sampler. Two mesh sizes, 28 and 10 μ, are available from G. M. Manufacturing Corporation, New York. Either meshs ize will retain the smallest of rotifers and nauplii. The 10-μ size will also trap many phytoplankton species, though it filters more slowly than the larger mesh.

Sampler operation — The upper and lower doors of the sampler swing upward to the vertical position as the sampler is lowered, with the lower

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[1] When not in use, the trap was usually carried in a fitted box lined with ¼-inch foam rubber, which helped to prevent scratching of the plexiglass. After a year's field work the transparency was still good.

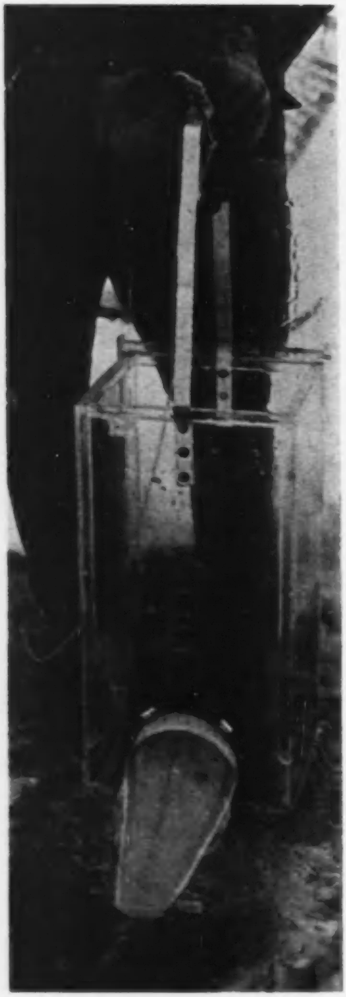

FIG. 3. The transparent trap in use during winter.

door blocking the mouth of the net (see Fig. 4). When the sampler is stopped, both doors swing gently shut, and seal tightly as the sampler is raised. Leakage is negligible; if the screened hole shown in the upper lid is not added, the seal is so tight as to keep water from running out through the net. As the sampler is pulled from the water its contents are filtered through the net, so that only the empty sampler is raised from the water. The contents of the net are easily rinsed into a sample bottle with the use of a wash bottle. No messenger is required and there are no tripping devices to set before lowering the sampler, so that several samples may be taken very quickly.

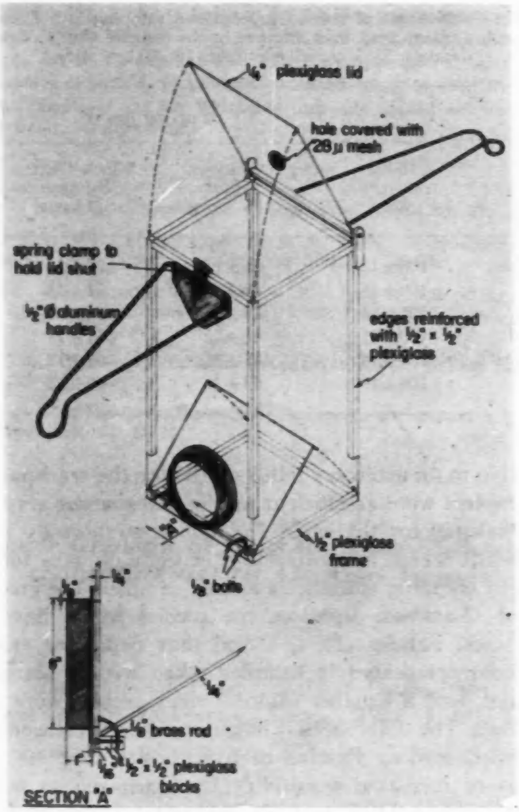
1/4" plexiglass lid
hole covered with 28 μ mesh
spring clamp to hold lid shut
1/2" Ø aluminum handles
edges reinforced with 1/2" x 1/2" plexiglass
1/2" plexiglass frame
1/8" bolts
1/4"
1/4"
1/8" brass rod
1/16"
1/2" x 1/2" plexiglass blocks
SECTION "A"

FIG. 4. Construction details for the transparent trap.

Sampler efficiency — The transparent sampler was tested against three other sampling devices which are currently very popular. The test was performed in FRB Experimental Lake No. 122 near Kenora, Ontario. The test procedure was similar to that designed by Patalas (1954).

At each of 10 stations two vertical series of 10 samples were taken at 1-m intervals using a 9-liter opaque van Dorn for one series, and the transparent trap for the other. All 10 samples taken with each device were pooled and considered to be one replicate. Two 10-m vertical hauls were also made at each station using a Clarke–Bumpus adapted for vertical tows for one haul and a 12"-diam metered townet for the other. The same procedure was repeated at all 10 stations. Replicate samples were concentrated in graduate cylinders and subsampled for counting. At least 100 animals of each species were counted from each replicate, except when there were fewer than 100 specimens of the species in the sample. Results are set out in Table 1. All

TABLE 1. Relative effectiveness of several zooplankton samplers. All figures are based upon an index of 100 for the transparent trap. Figures to the right of the ± sign represent approximately 95% confidence limits for 10 replicates.

Species	Sampling device			
	28-liter transparent trap	Opaque 9-liter van Dorn	5-inch Clark–Bumpus #15 net	12-inch metered townet #20 mesh
Holopedium gibberum	100±18	65±15	71±18	65±24
Daphnia sp.	100±14	62±18	74±13	60±30
Leptodora kindtii	100±26	54±41	35±24	59±33
Diaptomus leptopus	100±21	60±22	54±23	49±21
Diaptomus minutus	100±18	105±25	68±12	86±31
$\bar{x}$	100±19	69±24	60±18	64±28

results are relative to an arbitrary index of 100 for the transparent trap. None of the other samplers were as efficient as the transparent trap except for the high capture efficiency for the small *Diaptomus minutus* by the opaque van Dorn trap, a result which is inexplicable in light of data for other species. The differences in trapping efficiencies among samplers are greatest for *Leptodora kindtii* and *Diaptomus leptopus*, the species which have the strongest avoidance reactions. Patalas (1954) found that *Leptodora* and *Eudiaptomus gracilis* were underrepresented in samples taken with a plankton pump, an unmetered townet, and a Ruttner sampler when results were compared with his self-closing trap. The differences in efficiency were decreased when sampling was done at night, leading Patalas to hypothesize that either visibility of opaque samplers or increased sensitivity by Crustacea to water current in the light was the responsible factor. The presence of towing rope and bridles may be partly responsible for the reduced efficiency of the nets tested in this study, as both devices were towed directly from the front. Sampler capacity and relative aperture size of net or trap may also be important characteristics. It is likely that the transparent trap catches different species with different efficiencies, though this was not tested.

The sampler as shown has been tested only on small lakes. Much heavier materials should be used in construction of samplers to be used on large lakes. During rough weather on large lakes, the pitching motion of a ship may allow the sampler to drop back occasionally during ascent. It is conceivable that such action might allow the lower door of the sampler to open by forcing water out through the net. This possibility can be avoided by constructing a lid to close the net aperture during sampling, which can be removed at the water surface so that the sample may be filtered (see Fig. 4). Such a lid will also protect the net during transport.

Material for constructing the transparent trap costs approximately $60, including the nannoplankton net.

Acknowledgments — Mr C. Stuart designed the tripping mechanism for the syringe sampler and constructed both devices. Dr J. E. Nighswander and Mr Adrian Clark contributed helpful ideas to both designs. Dr K. Patalas and Dr J. R. Vallentyne offered helpful criticisms of the manuscript. Construction of both instruments was financed by a grant from the National Research Council of Canada. Testing of the self-activating trap was carried out with the assistance of Fisheries Research Board summer staff.

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Range Extension of Flag Rockfish (*Sebastodes rubrivinctus*) to Aleutian Islands[1]

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BEST, E. A., AND PETER J. ELDRIDGE. 1969. Range extension of flag rockfish (*Sebastodes rubrivinctus*) to Aleutian Islands. J. Fish. Res. Bd. Canada 26: 1955–1956.

During August 1967, 12 specimens of *Sebastodes rubrivinctus* were collected from the Pacific Ocean off Amchitka Island, Alaska. This is a westward extension of their known range from the Gulf of Alaska.

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THE FISHERIES RESEARCH INSTITUTE of the University of Washington, under contract from Battelle Memorial Institute, conducted a survey of the marine environment in the vicinity of Amchitka Island, Alaska, during the summer

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of 1967. On August 30, while engaged in bottom fishing, the chartered otter trawler *Paragon* collected 12 specimens of *Sebastodes rubrivinctus* from a depth of 220 fath (402 m) southeast of Amchitka Island (51°17′N, 179°27′E). The location of capture is considerably farther west than previously reported occurrences in the Gulf of Alaska. Single specimens have been recorded from southeast of the Copper River Flats, 60°02′N, 144°41′W (Heyamoto and Hitz, 1962), and southeast of Kodiak Island, 57°27′N, 150°20′W (Barsukov, 1964).

The fish were readily identified by their characteristic coloration of red stripes on a pink background. The identification was verified by the use of Phillips' (1957) key to the rockfishes. Nine of the fish were males and three were females, and their length measurements ranged from 266 to 472 mm. The majority of the fish in the same trawl haul were of the species *Sebastodes aleutianus*, and small numbers were of the following: *S. alutus*, *Sebastolobus alascanus*, *Atheresthes stomias*, *Glyptocephalus zachirus*, *Theragra chalcogrammus*, and *Malacocottus kincaidi*.

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Experimental Tank to Simulate Certain Reservoir Conditions

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PUGH, JOHN R., ALAN B. GROVES, AND WESLEY J. EBEL. 1969. Experimental tank to simulate certain reservoir conditions. J. Fish. Res. Bd. Canada 26: 1956–1959.

To simulate reservoir conditions, a test tank holding 17,624 gal was built. Made of three cylindrical 10-ft × 10-ft steel sections bolted together, it is equipped with 18 observation ports and numerous fittings that permit water sampling, treatment, and temperature control. Observations have been made on juvenile salmon in water supersaturated with nitrogen.

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INCREASED EVIDENCE of mortality of juvenile salmon in large reservoirs of the Pacific Northwest (Collins and Elling, 1964) created a need for a test facility in which certain chemical and physical reservoir conditions could be simulated and controlled. Personnel of the Seattle Biological Laboratory, U.S. Bureau of Commercial Fisheries, have designed and built a suitable cylindrical test tank (Fig. 1). According to Rees (1957) and Smith et al. (1968) most juvenile salmon remain near the surface of reservoirs, in depths less than 30 ft.

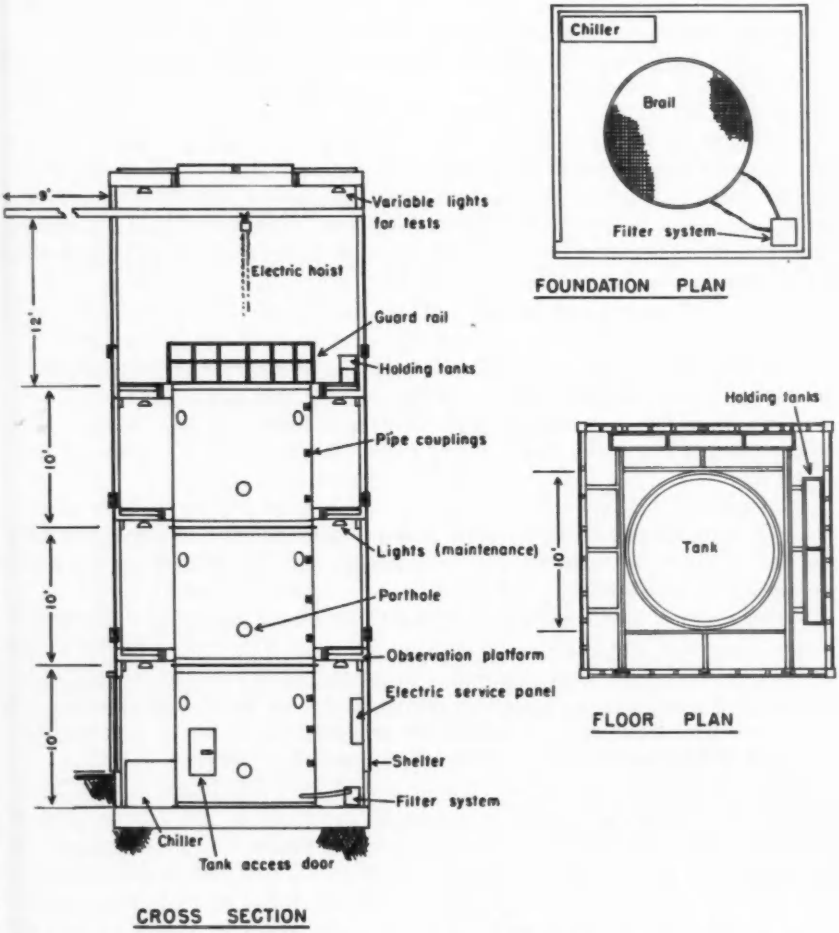
Chiller
Brail
Filter system
FOUNDATION PLAN
Holding tanks
10'
Tank
FLOOR PLAN
9'
Variable lights for tests
Electric hoist
12'
Guard rail
Holding tanks
10'
Pipe couplings
Lights (maintenance)
10'
Porthole
Observation platform
10'
Electric service panel
Shelter
Filter system
Chiller
Tank access door
CROSS SECTION

FIG. 1. Experimental tank to simulate certain chemical and physical conditions of reservoirs.

The tank has a volume of 2356 ft^3 and holds 17,624 gal. It is 10 ft in diameter and 30 ft deep, and is constructed of sheet steel ($\frac{1}{8}$-inch thick) in three cylindrical sections of 10 ft × 10 ft. All sections have a 4-inch flange at each end; the sections are fastened together with large bolts, evenly spaced around the flanges. A rubber gasket between the flanges of adjoining sections forms a watertight seal. A reinforced concrete slab, 18 ft^2 × 18 inches thick, serves as the supporting base.

Observation ports (10 inches in diameter) are arranged in six horizontal planes, with three ports at 120-degree intervals in each plane. The ports are staggered in successive planes, which are located 3, 6, 12.5, 17.5, 22, and 27.5 ft from the bottom. Three observation platforms at 10-ft vertical intervals enable an observer to view all areas within the tank.

Regular city water is supplied through a 2-inch line. The tank is equipped with a sand filter system capable of filtering 2520 gal per hour. At optimum circulation, all of the water is filtered about once every 7 hr.

Temperature control equipment consists of a "chiller" and three 10.5-kw heating elements. Temperature control is ±0.1 degree C. Rate of change varies with ambient air temperature and volume of water; within normal operating temperatures (10–27 C), however, the system is capable of heating the entire contents of the tank at a rate of 0.6 degree C per hour or of cooling at a rate of 0.1 degree C per hour.

Five small troughs (4 × 1 × 1 ft) on the top observation platform, supplied with dechlorinated city water, are used to hold fish before testing.

A brail facilitates removal of fish. The brail is rimmed with rubber to form a fish-tight seal against the walls of the tank.

The tank has an access door (18 inches × 3 ft) near the bottom; when it is empty, a researcher can enter to place his experimental apparatus or to clean the tank.

A wooden building encloses the tank, observation platforms, filter system, and temperature control equipment. A lifting device in the roof and two large doors near the top facilitate the installation and removal of experimental apparatus.

Illumination in the tank is provided by overhead lights, so that all light enters the water from above the surface. An automatic timing device allows simulation of a wide range of conditions, including sunrise and sunset. Each observation platform is equipped with lights for external maintenance, but these are darkened while tests are in progress.

Spaced around the side of the tank at about 2-ft intervals (6 inches from the bottom) are 16 fittings, 1–4 inches in diameter. Additional 1- and 2-inch fittings are provided on opposite walls, 20 inches from the bottom, and at 40-inch vertical intervals over the remainder of the tank. Two fittings of the same size are placed at each vertical location. These fittings accommodate the hoses of the filter system, provide for water sampling over the entire depth, accommodate the heating elements, and make possible water withdrawal for cooling or chemical treatment and return at the same or a different depth.

By installing heating elements at various levels or circulating the water from any depth through the cooling system or through other types of experimental apparatus, an investigator can simulate a variety of physical and chemical conditions that might occur naturally in a typical reservoir.

Recently, the tank was used to examine the effect of water supersaturated with nitrogen on the behavior of young chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*). A recirculation system capable of pumping 10,800 gal per hour was connected to the tank. The air intake of the recirculation pump was regulated until the nitrogen saturation in the tank reached 125%. Periodic sampling of the water allowed the researchers to monitor the gradual change in saturation of nitrogen. When the test fish were introduced, the investigators were able to observe their response to the nitrogen-saturated water and to visually establish vertical distribution patterns.

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Appraisal of the Iodate–Sulphuric Acid Wet-Oxidation Procedure for the Estimation of the Caloric Content of Marine Sediments

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HUGHES, ROGER N. 1969. Appraisal of the iodate–sulphuric acid wet-oxidation procedure for the estimation of the caloric content of marine sediments. J. Fish. Res. Bd. Canada 26: 1959–1964.

Endothermic reactions of the inorganic fraction of marine sediments and the low organic matter content invalidate the determination of caloric values by bomb calorimetry. However, oxidation of the organic fraction with excess potassium iodate and back titration with sodium thiosulphate provides a suitable method for estimating the caloric value.

Contrary to earlier reports, a correction factor for the incomplete combustion of proteins, derived from the Kjeldahl nitrogen content of the sample, must be applied. Interference by the inorganic fraction of the sediment in the titration procedure is allowed for by a control flask containing a sample of the sediment from which the organic matter has been removed by heating in a muffle furnace. Any ferrous sulphide in the sample is oxidized prior to digestion.

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IN STUDYING the ecological energetics of detritus-feeding benthic animals it is important to measure the caloric content of the mud before and after ingestion. These values, in conjunction with rates of feeding and defaecation, are used in estimating the rate of assimilation of energy by the population. During productivity studies of *Scrobicularia plana* (da Costa), a bivalve feeding directly on sediments, it was found impossible to estimate the energy content of the food or faeces by bomb calorimetry. Combustion of mixtures of sediment with benzoic acid resulted in a net decrease in total caloric value due to the endothermy of the inorganic fraction of the sediment, probably caused by loss of carbon dioxide from carbonates and water from clay minerals. An attempt was made to overcome this problem by adopting the wet-oxidation procedure, modified after Karzinkin and Tarkovskaya (1964), using potassium iodate in sulphuric acid. These authors had claimed complete oxidation of all organic matter in small samples of organisms used as fish food, thus improving on the dichromate wet-oxidation procedure used by various authors (Ivlev, 1935; Riley and Skirrow, 1965; el Wakeel and Riley, 1957; Kay, 1954; Strickland and Parsons, 1965), in which an arbitrary correction for incomplete oxidation of protein is necessary based on the Kjeldahl nitrogen content of the sample. However, my results showed that the oxidation of protein by the iodate method was also incomplete, so that a correction of the same kind was necessary even with this method. Nevertheless, with certain precautions the iodate method proved suitable for estimating the caloric contents of marine sediments and faecal pellets.

In any wet-oxidation method for measuring caloric content, unless the oxidising agents react stoichometrically with the organic material, some arbitrary relationship must be assumed which may differ for different types of organic material. It is very desirable, therefore, that the method should be checked by comparing the results from wet-oxidation procedures with those obtained from direct calorimetry of the same material.

In this paper modifications of the iodate–sulphuric acid method are described which have made it satisfactory for the purpose of measuring assimilation by detritus-feeding organisms.

Methods — Samples of dry sediment (0.2–0.3 g) were weighed accurately into 100-ml Kjeldahl flasks, to which were added 5 ml of 2 N sulphuric acid. Air was bubbled vigorously through the liquid for ½ hr to drive off any hydrogen sulphide derived from ferrous sulphide which may have contaminated the sample. Sulphuric acid (20 ml) (sp gr 1.84) and 3 ml of potassium iodate (5% w/v) were then added to the flask. A control flask contained the above reagents together with a sample of sediment (0.5 g) which had been heated to 500 C in a muffle furnace to remove all organic matter. The flask contents were boiled for 1 hr during which time free iodine was liberated. After cooling, the digests were diluted to about 50 ml and the flasks were immersed in a boiling water bath. Air was bubbled through the digests to remove the free iodine and heating was continued until the digests were a clear yellow–green; any traces of a brownish colouration indicate that all the free iodine has not been removed. The digests were again cooled and diluted to a standard volume (250 ml). Aliquots (10 ml) were then pipetted into titration vessels containing 10 ml of potassium iodide solution (15% w/v). The excess iodate liberated free iodine which was titrated against standard sodium thiosulphate (0.1 N), using sodium starch glycollate as indicator. The sample titres were subtracted from the control titre to give values corresponding to the iodate used in the oxidation. It follows from the equation:

$$6\ KIO_3 + 3\ H_2SO_4 = 3\ K_2SO_4 + 3\ H_2O + 6\ I + 15\ O$$

that 1 ml of 0.1 N thiosulphate, which is equivalent to 3.567 mg of iodate, is also equivalent to 0.6667 mg of oxygen, since 1 mg of iodate corresponds to 0.1869 mg oxygen. The caloric value of the sample can be calculated by assuming that when 1 mg oxygen is used to burn organic matter, 3.38 cal are released (Ivlev, 1935). Therefore, using the oxy-caloric coefficient of 3.38, the caloric content of the sample is given by the relationship:

$$\text{caloric content per g dry weight} = \frac{t \times 0.6667 \times 3.38 \times 25}{W}$$

where t is the titre difference of 0.1 N thiosulphate in milliliters, and W is the dry weight of the sample in grams.

Results and discussion — Sediment samples of different specific organic matter content were obtained by mixing together the appropriate proportions of untreated sediment and sediment previously heated in a muffle furnace for 2 hr at 500 C to remove organic matter. Figures 1A and 1B show the linear relationship between total organic matter content and caloric value for samples of different weight and different specific organic matter content.

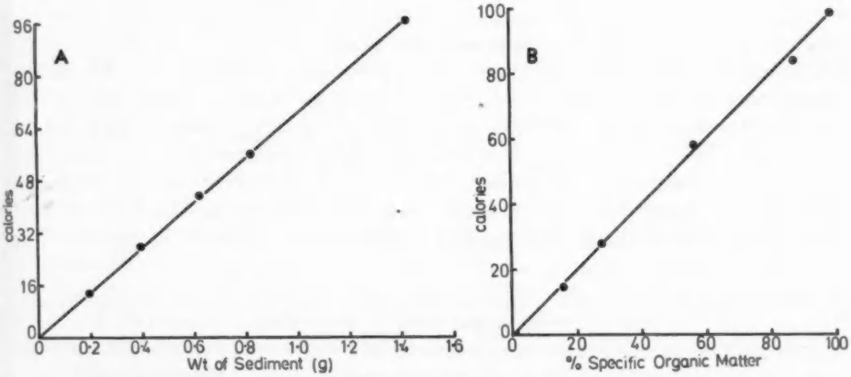

FIG. 1. A, Relationship between caloric content determined by wet-oxidation and weight of sediment. B, Relationship between caloric content determined by wet-oxidation and specific organic matter for samples of equal weight and containing various proportions of organic-free sediment. Samples of organic-free material were obtained by heating the sediment in a muffle furnace.

The use of control flasks containing organic-free sediments relates to the finding that such sediments, digested in the absence of iodate, themselves liberate iodine from potassium iodide, and give rise to a positive titre against thiosulphate. The relationship between weight of organic-free sediment and thiosulphate titre is illustrated in Fig. 2 for sediments from two different localities. Thus far it has been assumed that oxidation of the organic matter

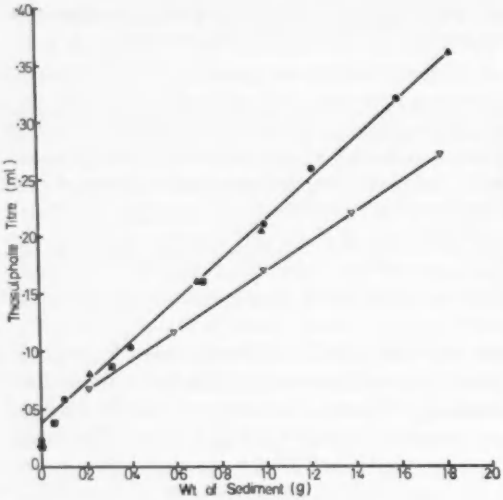

FIG. 2. Relationship between thiosulphate titre and weight of organic-free sediment digested in the absence of potassium iodate. Sediment samples obtained from: locality 1 — Menai Straits, Anglesey. ● = sample A — Mud; ▲ = sample B — Mud, taken 100 yards from A; ■ = sample C — Faeces from *Scrobicularia* feeding at this locality; locality 2 — Aber Foreshore, Caernarvonshire. ▽ = sample D — Muddy sand.

content of the samples was complete and that the coefficient of 3.38 cal/mg O_2, taken from the report of Ivlev (1935), who gave values for the oxy-caloric coefficients of protein, fat, and carbohydrate as 3.305, 3.280, and 3.529 cal/mg O_2 respectively, can be applied directly. However, when caloric values for samples of protein, carbohydrate, and lipid, obtained by wet oxidation with iodate, have been compared with values obtained by standard bomb-calorimetry (Table 1), good agreement between the two methods was found only with carbohydrates and lipid. Evidently wet-oxidation of protein was

TABLE 1. Comparison of caloric values for carbohydrate, lipid, and protein, obtained by wet-oxidation and bomb calorimetry.

Sample	Oxy-caloric coefficient	Wet-oxidation[a]			Bomb-calorimetry		
		Kcal/g	SE	n[b]	Kcal/g	SE	n
Benzoic acid	3.212	6.2619 (6.319)	0.05	3	6.319[c]		
Cellulose	3.529	3.9702	0.03	4	3.9431	0.02	4
Glycogen	3.529	3.7625	0.02	4	3.7373	0.04	4
Olive oil	3.280	9.3723 (9.6580)	0.02	5	9.3865	0.02	5
Bovine albumin	3.305	4.8300 (4.9395)	0.03	7	5.8087	0.01	4

[a] Figures in parentheses refer to caloric values calculated using a mean oxy-caloric coefficient of 3.38 (see text).
[b] n = number of replicate samples.
[c] British Chemical Standard.

incomplete under the conditions employed, giving a lower value for the caloric value. This contrasts with the earlier report of Karzinkin and Tarkovskaya (1964) which implied that wet-oxidation of proteins by iodate is essentially complete. In the analysis of unknown samples a correction factor must therefore be applied. The protein content of the sample is estimated from the Kjeldahl nitrogen content which must be determined separately. If fully oxidized, the average caloric value for proteins is taken to be 5.65 Kcal/g (Maynard and Loosli, 1956). Assuming 16% of the protein exists as Kjeldahl nitrogen and for routine analysis that wet-oxidation of proteins is 80% complete (Table 1), the caloric value of unoxidized protein in terms of Kjeldahl nitrogen is 0.0071 Kcal/mg Kjeldahl nitrogen. Undoubtedly the exact percentage oxidation of different protein types will vary slightly. The results in Table 2 show the application of the correction factor for wet-oxidation by iodate to data for a sample of *Scrobicularia plana* tissue, expressed as Kcal/ash-free gram, and compare it with direct bomb-calorimetry. The corrected value is remarkably close to the value obtained by direct calorimetry but such good agreement is probably fortuitous. Analysis of four samples of *Scrobicularia* tissue in the presence of sediment gave recoveries of organic matter content of 96.9 ± 1.8%.

TABLE 2. Wet-oxidation of *Scrobicularia* tissue: application of a correction factor for incomplete oxidation of protein.

Oxy-caloric coefficient	% protein from Kjeldahl nitrogen	Correction factor	Caloric content wet-oxidation		Bomb-calorimetry
			Observed	Corrected	
3.38	57[a]	0.6441[b]	4.4611	5.1502	5.1020

[a] Determined by auto-digestion in a Technicon automatic Kjeldahl nitrogen analyser.
[b] Assuming 80% digestion, and a mean value for protein of 5.65 Kcal/g (see text).

Ferrous sulphide in the digestion mixture reduces potassium iodate with consequent effect on the final titration value. A similar interference by sulphide on the dichromate wet-oxidation procedure was reported by Southward (1952). This problem was overcome by treating the sample with 5 ml of 2 N sulphuric acid prior to digestion. Air bubbled through the liquid drives off the hydrogen sulphide, although it is possible that iron still exists in the ferrous state. However, after such treatment, samples to which had been added 5% by weight of ferrous sulphide gave titration values similar to those of uncontaminated samples of the same sediment (Table 3). Alternatively the ferrous sulphide which contaminates certain sediments may be oxidized by heating in a vacuum oven at 50 C for 12 hr with the air inlet valve open. The samples are dried to constant weight by stopping the air flow and heating for a further 12 hr in vacuum (c.f. Newell, 1965). It is assumed that no change in chloride concentration occurs during treatment of the sediment in the muffle furnace

TABLE 3. Samples treated by mixing with 5 ml of 2 N sulphuric acid and bubbling air through the liquid for ½ hr prior to digestion.

	Sediment	Sediment + 5% dry wt of FeS
Titre (ml thiosulphate)	1.500 1.510 1.495	1.505 1.500 1.510

and that any interference by chlorides with the wet-oxidation is corrected by the blank titration.

The wet-oxidation of marine sediments with potassium iodate in sulphuric acid is shown to be suitable for estimating their caloric contents if allowance is made for the incomplete oxidation of protein and for the interference of inorganic material with the titration procedure. As in other wet-oxidation methods, the iodate procedure does not distinguish between readily assimilable carbon compounds and nonassimilable matter such as coal, lignin, etc. (Southward, 1952). However, the method remains valid for comparisons between the input and output of animals feeding directly on sediment since nonassimilated oxidisable material will be present in both. For a distinction between assimilable and nonassimilable fractions of oxidisable matter the method devised by George (1964) or similar techniques may be considered.

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Hermaphrodite Specimen of the Sand Dollar, *Echinarachnius parma*

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HEROLD, RICHARD C. 1969. Hermaphrodite specimen of the sand dollar, *Echinarachnius parma*. J. Fish. Res. Bd. Canada 26: 1965–1966.

This is the first report of an hermaphrodite in the sand dollar *Echinarachnius parma* (Echinodermata). It was collected off Swans Island, Maine, during July 1968. The animals' gonads consisted of one female gonad and four ovotestes. Self-fertilization did not occur between the artificially shed eggs and sperm, probably because the eggs were not fully ripe. The sperm fertilized eggs from other animals. The discovery of an hermaphrodite in this species has important implications for its use in embryological experimentation.

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THE SAND DOLLAR, *Echinarachnius parma* (class Echinoidea), is extensively used as a source of eggs and sperm for embryological experimentation at marine biological centers along the northeast Atlantic coast of North America. In a group of sand dollars obtained during the summer of 1968, I observed an hermaphroditic specimen, which upon being peristomially injected with 0.5 KCl (Tyler, 1949) shed both eggs and sperm. This is the first report of an hermaphrodite in *Echinarachnius parma*. Echinoids are strictly dioecious and hermaphrodites occur only as rare anomalies (Hyman, 1955). Hermaphrodites have been reported and discussed in other species of sea urchins and sand dollars (Boolootian and Moore, 1956; Delavault, 1966; Harvey, 1956a, b; Hyman, 1955).

The discovery of a distinct hermaphrodite in this species has implications for its use in embryological work, because many experiments are based on the ability to control initiation of fertilization. Hermaphroditic animals containing only small portions of the gonads of the opposite sex could occur whose shed gametes would appear normal macroscopically. This might explain the spontaneous cleavages occasionally obtained in unfertilized egg samples.

The hermaphrodite was obtained in a dredge collection taken on July 29, 1968, from a bed located north of Great Baker Island at 68°25′45″W and 44°07′30″N near Burnt Coat Harbor, Swans Island, Maine. At low water approximately 6–10 ft of water covered the bed. Water temperature was 12 C.

The hermaphrodite was first noticed because of the unusual orange color of its shed gametes. The sexes in this sand dollar are ordinarily separate and the shed gametes are quite distinct. The eggs are red–purple due to the presence of echinochrome granules in the jelly coats, and the sperm is cream color. There are no external morphological characteristics which allow sexing of the animals so that gamete color must be observed to determine sex. The sex ratio is approximately 1:1.

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Microscopic examination of the shed gametes of the hermaphroditic animal showed both sperm and eggs were present. Eggs and sperm each occupied about one half of the total volume of the sample. The unusual color of the shed gametes was probably due to the blending of normal gamete colors, since this color could be simulated by mixing one-third normal eggs to two-thirds normal sperm on a volume basis.

The female gametes from the hermaphrodite appeared normal, i.e. they were of normal size, the cytoplasm was finely granular, the germinal vesicles had broken down, and the vitelline membranes and the echinochrome granules included in the jelly coats looked normal. The sperm were of usual comma-shape size and showed normal swimming activity.

Self-fertilization did not occur. Neither fertilization membrane lifting or cleavage occurred. In most of the other hermaphrodite sea urchins reported, self-fertilization did occur (Harvey, 1956a). Lack of self-fertilization between the gametes of this specimen was probably due to unripe eggs. Sperm from the hermaphrodite fertilized eggs from other animals.

The anatomy of the hermaphrodite, as would be expected in an animal which does not have external sexual dimorphism, showed no unusual external morphological characteristics. The diameter of the test was 55 mm which indicated an age of 2–3 years.

The internal morphology was examined by dissecting the animal from the oral surface. All internal organs with the exception of the gonads appeared normal. Gonads were examined microscopically for the presence of eggs and sperm. The five gonads were identified by numbers 1 through 5 starting with number 1 in the immediate counter-clockwise position from a line between the mouth and anus. Gonad 1 consisted entirely of ovary tissue and 2–5 were ovotestes. The second gonad consisted mostly of ovarian tissue but contained a small central mass of testicular tissue. The third gonad was made up mainly of testicular tissue with a few small scattered masses of ovarian tissue. The fourth and fifth gonads showed approximately equal amounts of ovarian and testicular tissue, these being fairly intimately mixed.

Hermaphrodites appear to be rare in *E. parma*. Four to five hundred specimens from the same bed population were examined without finding any other hermaphrodites. The past several summers over 2000 sand dollars from many different beds have been shed for gametes; no hermaphrodites were found.

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